The form and function of avian rictal bristles

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The form and function of avian rictal bristles

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Abstract

Although mechanoreception is present throughout the animal kingdom, it is still relatively under-studied and poorly understood, especially in nocturnal birds. A particular type of facial feather, the rictal bristles, are thought to carry out a similar tactile function to mammalian whiskers, of which they superficially resemble. If they do, such a function could enhance foraging behaviour and facilitate obstacle avoidance, especially in dark, complex habitats. However, as rictal bristles are the least described of any feather, little is known about them. Therefore, this thesis characterises rictal bristle form and function by describing: i) bristle morphology and follicle anatomy, ii) the development of rictal bristles, and iii) the associated mechanosensory brain areas. It will also explore: iv) rictal bristle evolution and v) function. Results in this thesis demonstrated that rictal bristle morphology and the presence of mechanoreceptors around the follicle varied between species. Specifically, diurnal species did not have mechanoreceptors around their bristle follicles and had shorter bristles. Associated mechanosensory brain areas also varied between species, but there was no clear association between the neuroanatomy, rictal bristle morphology or foraging traits. Rictal bristles were absent in two species of altricial hatchlings, and only emerged after their eyes opened. Stimulation of the rictal region in these chicks led to behavioural feeding responses, especially coinciding with when chicks started to feed independently. Rictal bristle evolution underwent multiple events of disappearance and gain during avian evolution, and therefore, the presence and morphology of the rictal bristles also varied between orders, families and genera. Short rictal bristles with barbs at the base were likely to be present in the common ancestor of the phylogeny (108 mya). Rictal bristle presence and length were associated with nocturnality and foraging methods, and diet is also likely to be associated with rictal bristle length. Consequently, this thesis suggests that, in adult birds, rictal bristles are likely to act as facial tactile sensors in species that forage in low-light conditions in complex habitats. Rictal bristles are may, therefore, play a role in collision avoidance, foraging and eye protection. Species foraging in the daytime might have rictal bristles with a reduced tactile function. However, identifying rictal bristle function is challenging and demands further investigation. This thesis provides the first comparative description of avian rictal bristle form and function, and is an important foundation for further investigation of the sense of touch in birds.

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Table of Contents

Notes for this thesis

Throughout the thesis, figures will be written as Figure 1-4a (for example). This incorporates the figure panels, which are alphabetized (a), the figure numbers (4), and the chapter number (1).

Chapter 1

Introduction and overview

Facial sensors in the animal kingdom

Chapter summary:

The research presented in this thesis focuses on the form and function of avian rictal bristles, examining, specifically, their role as tactile facial sensors. While rictal bristles are present in many avian species, their comparative form and function are almost entirely undocumented. Tactile facial sensors are common and occur across the animal kingdom. Mammalian whiskers are perhaps the most well-described facial sensor, and most closely resemble avian rictal bristles, in terms of their form and location. Therefore, research in this thesis is inspired by mammalian whisker studies and formulates hypotheses based on what we already know about mammalian whiskers. Furthermore, it applies Tinbergen's approach to structure these hypotheses. Therefore, the thesis will systematically investigate rictal bristle anatomy, development, evolution and function. This chapter will justify my approach and introduces the scientific foundation of the thesis by: i) introducing facial sensors, ii) providing an overview of mammalian whisker research, and iii) demonstrating the lack of information on rictal bristle form and function. It concludes by describing the aims and objectives of the thesis.

1.1 An overview of facial sensors in the animal kingdom

Mechanosensation gives animals the ability to perceive vital information from their surroundings, by either direct touch, displacement, tension, pressure or vibration (Prescott *et al.*, 2016). Many animals have facial sensors that are associated with touch in some way, including barbules in fish, antennae in arthropods, whiskers in mammals, and perhaps even rictal bristles in birds. Although their form and function vary considerably.

In fish, certain species (e.g. Mixini, Chondrostei, many Teleostei and some Chondrichthyes) possess pairs of epidermal outgrowths that extend downward from the lower jaw near and around the mouth, termed barbels (Figure 1-1) (Kasumyan, 2011). Barbels are mechanosensitive and have numerous tactile receptors across their epidermis (Kasumyan, 2011) allowing fish to discriminate food from substrates by probing the loose materials on the bottom floor (Singh and Kapoor, 1967; Prescott et al., 2016). However, barbels also contain chemosensory receptors (Agarwal and Rajbanshi, 1965; Bone and Moore, 2008), therefore they play a dual sensory role. Barbels vary in number, position, mobility, morphology (relative length and shape) and anatomy (e.g. presence/absence of cartilaginous axial rods and taste buds) across taxa (Figure 1-1) (Kapoor and Bhargava, 1967; Singh and Kapoor, 1967; Kasumyan, 2011). Barbel form is therefore diverse across species, suggesting that their function is also diverse and likely to be related to the fish anatomy, behaviour, feeding ecology, and the development of its other sensory systems (Figure 1-1) (Schmidt, 1983; McCormick, 1993; Kasumyan, 2011). Indeed, barbels tend to be more prominent when the fish environment or feeding conditions are darker (e.g. with turbidity), limiting visual orientation (Schmidt, 1983; Kasumyan, 2011). Barbel size were inversely related to the eye size in fish that use chemoreception to feed in murky waters, such as in great plains lotic (*Hybopsis spp.*) (Moore, 1950). However, the tactile role of the barbels, which plays an important role in food localisation (Kiyohara et al., 2002), does not seem to account for much barbel variation, e.g. some barbels do not protrude far beyond the mouth and protrude laterally rather than ventrally (e.g. longnose dace, *Rhinichthys cataractae*). Therefore, it seems unlikely that they are contact gustatory organs (Schmidt, 1983), and are more involved in chemosensing. Schmidt (1983) has proposed that barbels could be involved in i) orientation by detection of water-borne chemicals, and more specifically near-field prey location at night; ii) communication by reception of intraspecific chemicals, e.g. pheromones communication as the development of nuptial barbels in bluntnose minnows (*Pimephales notatus)*; or iii) communication by reception of interspecific chemicals, e.g. protection or evasion response to chemical “danger” signal released from damaged skin (termed, the schreckstoffe response).

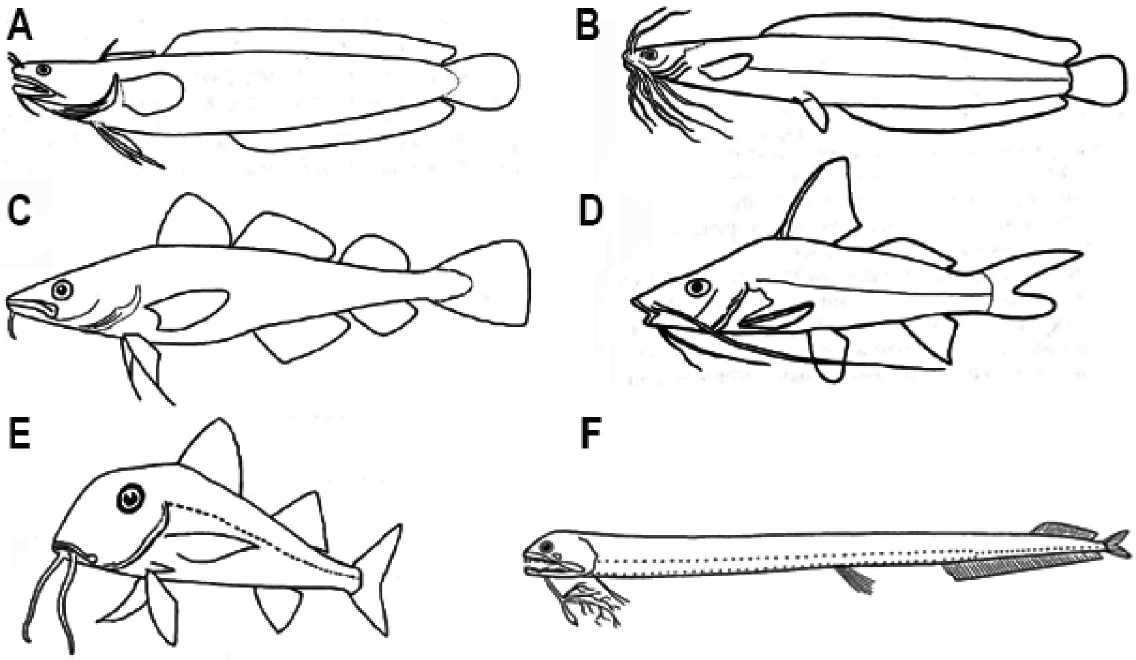


Figure 1-1. Different barbel morphologies present in various fish species: **A)** rockling (*Gaidropsarus vulgaris*), **B)** catfish (*Clarias spp.*), **C)** Atlantic cod (*Gadus morhua*), **D)** *Pimelodus spp.*, **E)** mullet (*Muls barbatus*), and **F)** dragonfish (*Eustomias satterleei*). Modified images from Kasumyan (2011).

In arthropods, tactile sensors are present in myriapods, crustaceans and insects, which have a well-developed, articulated pair of sensory appendages, the antennae, on their head (Figure 1-2C) (Staudacher *et al.*, 2005). Antennae are a mobile compound sensory organ, comprised of various sensory receptor types, including olfactory, chemo-, hygro-, thermo- and mechano-sensors (Schaller, 1978; Tichy, 1979; Tichy and Loftus, 1983, 1990; Shields and Hildebrand, 1999). Antennae are equipped with four types of mechanosensory structures that can be arranged in groups or as single receptors: hairs, campaniform sensilla, chordotonal organs, and stretch receptors or strand organs (Staudacher et al., 2005). These receptors relay proprioceptive (e.g. sensory hairs) and exteroreceptive (e.g. chordontal organs, external hair plates, campaniform sensilla) information, and play a role in locomotion and exploration by sensing, at close range, the position, shape and texture of surroundings objects, as well as recognising conspecifics and detecting predators (Staudacher et al., 2005; Prescott et al., 2016). Antennae are densely covered with different types of mechanosensory hairs, termed sensilla, which vary in shape, length, structure and type (Figure 1-2) (Schneider, 1964; Esslen and Kaissling, 1976; Toh, 1977, 1981; Altner and

Prillinger, 1980; Staudacher et al., 2005). Sensilla are able to move in their flexible sockets and can transduce both chemosensory information via several chemoreceptors cells, and mechanosensory information via a unique cell, or neuron, attached to the base of the hair (Staudacher *et al.*, 2005). Variation in the presence of sensory structures and their position on the antennae are likely to be associated with a species morphology, anatomy, ecological traits and behaviour, i.e. they are suited to sense specific stimulus or enable species-specific tasks, which have a behavioural significance to that species (Staudacher et al., 2005). For instance, the number of campaniform sensilla on the antennae flagellum per annulus vary across species of the genus *Apis* (honey bees). The sensilla number is higher on the honeybee flagellum, especially at its tip where the concentration is the highest, which is most probably related to their use of their antennae, e.g. comb building (Martin and Lindauer, 1966; Staudacher et al., 2005).

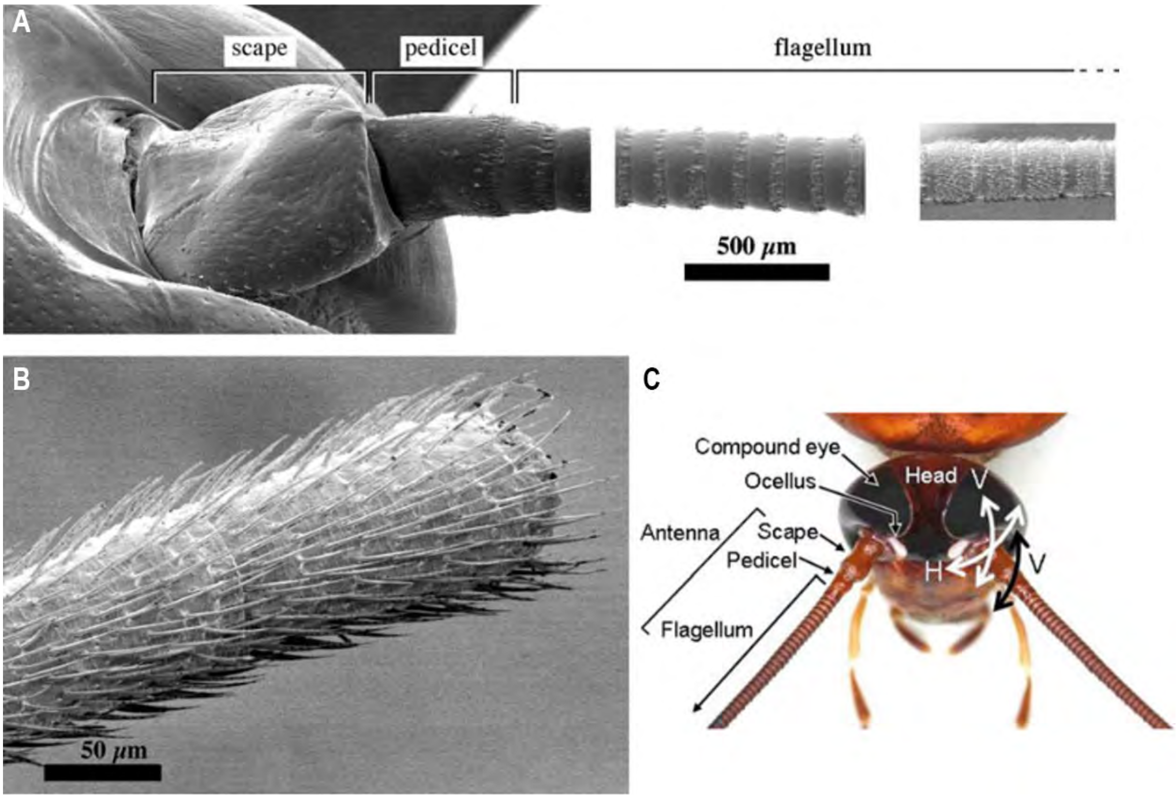


Figure 1-2. Insect antennae illustrating the sensory structures of their antennae. A) Different sections of the cricket antennae: scape, pedicel, and first two annuli of the flagellum (SEM picture), **B)** tip of the flagellum, **C)** Head and antennae of the American cockroach (*Periplaneta americana*). Arrows represent the horizontal (H) and vertical (V) movement capable by the antennae. Modified images from Staudacher *et al.* (2005) and Prescott *et al.* (2016).

Most mammals have tactile facial sensors, termed whiskers (Muchlinski, 2010). Whiskers are longer and thicker than mammalian pelage hairs (Figure 1-3) (Ahl, 1986). They project outwards and forwards of the snout in many mammalian species (Figure 1-3) (Prescott *et al.*, 2016). Whiskers are highly specialised in nocturnal terrestrial (e.g. rodents), tree-climbing (e.g. marsupials) and aquatic mammals (e.g. pinnipeds, manatees, otters) (Figure 1-3) (Roth-Alpermann and Brecht, 2009; Mitchinson *et al.*, 2011; Grant *et al.*, 2013; Milne and Grant, 2014). Indeed, they vary in structure across species, from atrophied whiskers in Ursidae (bears) to well-developed hairs in Sirenians (manatees and dugongs) and Pinnipeds (sea lions (Figure 1-3A), seals (Figure 1-3B), and walruses); therefore they have different degrees of specialisation (Murphy, 2013). Mammalian whiskers have a wide range of functional roles. They are used to guide orientation and locomotion, as well as object distance, position and shape discrimination (Brecht et al., 1997; Mehta et al., 2007; Sarko et al., 2011; Grant et al., 2018). Mammalian whiskers are contained by well-innervated follicles, termed the follicle-sinus complex, which contains different types of sensory receptors, including Merkel endings, Pacinian and Ruffini corpuscles (Rice et al., 1986; Muchlinski, 2010). Beneath the whisker follicle, many mammals have a network of intrinsic muscles, which give their whiskers mobility (Dörfl, 1982; Grant et al., 2013b), and many species can cyclically move their whiskers in a rhythmic sweeping back and forth motion, which is termed whisking (Dörfl, 1982; Haidarliu *et al.*, 2010, 2021; Grant *et al.*, 2013; Grant *et al.*, 2017) and occurs at high speeds of 5-25 times/second in rodents (Prescott et al., 2009). In rats, whisking movements generally occurs in bouts of variable duration (3-25 whisks/second) (Prescott et al., 2011) lasting 1 to 10 seconds (Hill et al., 2008). Although whisking frequency on both side is symmetrical and constant within a bout (5-7 Hz) (Gao et al., 2001; Evans et al., 2019), whisking amplitude can be asymmetrical, with whisking amplitude occurring within a range from ∼10 to 100°, at an average protraction velocity of ∼1000°/sec (Evans et al., 2019). Whisking, therefore, enables animals to actively scan their environment, collecting crucial tactile information to rapidly adapt their behaviour to their environment (Mehta et al., 2007; Mitchinson et al., 2011). For example, whisker movements can change in response to a contact within 13 ms (Mitchinson et al. 2007), indicating the rapidity of behaviours associated with whisker touch sensing.

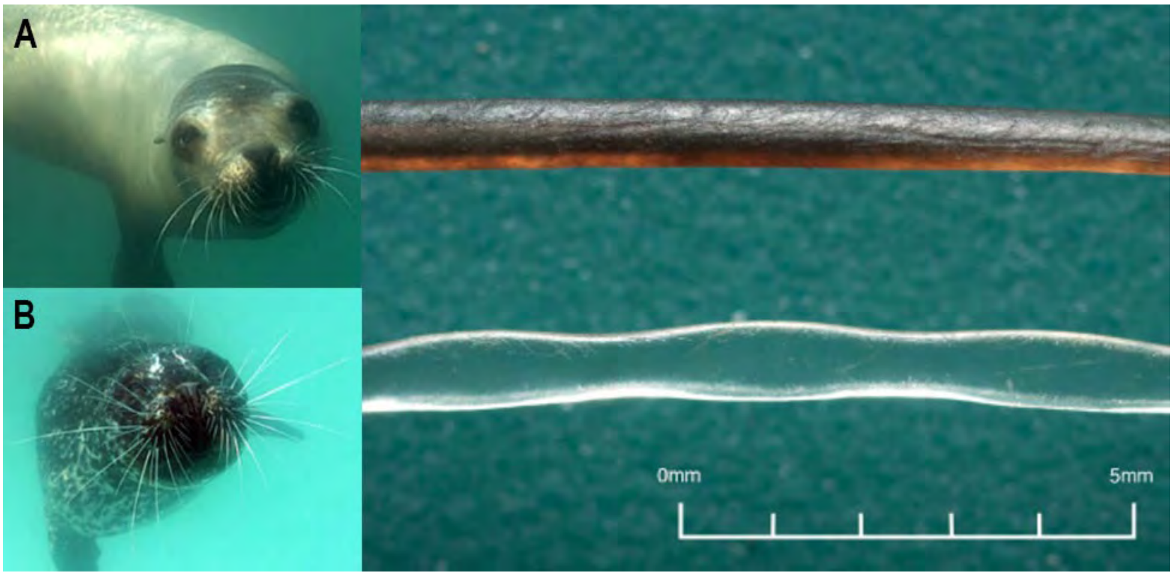


Figure 1-3. **Mammalian whisker shape** illustrated in A) California sea lion (*Zalophus californianus*) and B) harbour seal (*Phoca vitulina*). The two mammalian whisker shape presented are a smooth and an undulated profile. Modified images from Murphy (2013).

In birds, rictal bristles are hair-like feathers, present on the upper beak region of many species, such as the Caprimulgidae (Figure 1-4), Aegothelidae, Mimidae, Accipitridae and Tyrannidae (Lederer, 1972; Persons and Currie, 2015). Rictal bristles have a hair-like shape and are fairly stiff and tapered (Figure 1-4). Some bristles have barbs on the rachis, and all bristles are contained within an innervated follicle (Lederer, 1972; Pass, 1989). Evidence of mechanoreceptors, i.e. Herbst corpuscles, in the immediate surroundings of rictal bristle follicles have been found in some owls (*Bubo bubo, Asio flammeus, Athene noctua, Strix aluco*) and kiwis (*Apteryx spp.*) (Küster, 1905; Cunningham, 2010). Rictal bristles are present both in aerial feeders (e.g. Caprimulgidae and Tyrannidae), and ground feeders (e.g. Apterygidae) (Lederer, 1972; Cunningham et al., 2011). They are thought to protect the eyes (Corfield *et al.*, 2014), although their function is still largely unknown.

Figure 1-4. Photograph of the European nightjar (*Caprimulgus europaeus*) illustrating the rictal bristle position and shape**.**

As yet, not much is known about rictal bristle form and function; therefore inspiration from another body of work is needed in order to develop hypotheses. Whiskers in mammals are the most well-studied facial sensor system. Furthermore, they are probably the most similar tactile facial sensor to rictal bristles since: i) they are positioned similarly on the face, ii) they are organised as multiple singular entities (i.e. hairs or bristles), and iii) they are essentially dead cells within an innervated follicle. Therefore, this thesis will use inspiration from mammalian whiskers to develop a number of hypotheses around rictal bristle form and function. This chapter will next focus on whiskers, and provide an overview of what we know so far.

1.2 Mammalian whiskers

1.2.1 Whiskers have specialised morphology and anatomy

Whiskers are tapered hairs, composed of keratin and enclosed in large and highly innervated follicles (Rice et al., 1986; Voges et al., 2012; Belli et al., 2017). The tapered shape of the whiskers gives them more flexibility at the tip, which is thought to improve tactile sensing abilities, particularly in the discrimination of textures (Williams and Kramer, 2010; Hires et al., 2013). The cross-sectional shape of the whisker can vary between species, from circular to oval (Ginter *et al.*, 2010, 2012), and from smooth (e.g. all terrestrial mammals) to undulating along the length (e.g. phocid seals) (Figure 1-3) (Ginter *et al.*, 2010, 2012; Hanke *et al.*, 2010). Relative whisker length, taper, width and stiffness also varies across species, notably between larger aquatic species and smaller scansorial species; such that large, aquatic species have short, highly tapered, wide, stiff whiskers, which is thought to keep them rigid in the water for underwater sensing (Dougill *et al.*, 2020). Whisker follicle anatomy also differs, with many aquatic species having a 3-part follicle, which is thought to insulate the whisker for efficient sensing in cold waters (Dehnhardt et al., 1999). The number of nerve fibres around the whisker follicles also varies, and is much greater in aquatic mammals, especially in pinnipeds (Hyvärinen, 1989; Marshall *et al.*, 2006). Mechanoreceptors around the follicle sense the bending forces of the whiskers when they are in contact with a surface, and transmits the information in to neural signals to the brain (Diamond et al., 2008; Campagner et al., 2018; Evans et al., 2019).

Whisker arrangement tends to be ordered and follows a grid system, where whiskers are positioned in rows and columns in the mystacial pad of most mammals (Woolsey *et al.*, 1975), or a single row in cetaceans (Yablokov et al., 1972; Czech-Damal et al., 2012). The level of organisation varies amongst species, from highly organised in nocturnal, arboreal and aquatic mammals to less-organised in diurnal visual mammals (e.g. horses and deer) (Muchlinski et al., 2013, 2020; Grant et al., 2017). Whiskers of the same row move together due to the intrinsic muscles, which form a sling around each of the follicles (Dörfl, 1982). The intrinsic muscle architecture is conserved from marsupials to primates, but the exact arrangement can vary, such that diurnal species with less-organised whiskers tend to have disorganised and thinner intrinsic muscles (Muchlinski *et al.*, 2020). Complex movements of the whiskers, such as changing whisker symmetry, speed, spread and orientation, are controlled by extrinsic muscles sitting external to the mystacial pad, which vary greatly across species (Yohro, 1977; Grant et al., 2013a). These complex whisker movements are especially observed during object exploration (Mitchinson et al., 2007, 2011; Grant et al., 2009), which suggests that whiskers are active touch sensors.

1.2.2 Whiskers are prominent in the development

In rats and mice, whiskers follicles appear in embryo and whiskers are present at birth (Landers and Zeigler, 2006). Indeed, whiskers are present before ear-opening and eye-opening in both rats and mice (Grant *et al.*, 2012), perhaps indicating the importance of whisker sensing in young mammals. Rat pups start moving their whiskers with small unilateral retraction movements during their first postnatal week (Grant *et al.*, 2012) and whisker motions emerge gradually alongside their locomotion abilities, becoming established by P17 (Grant *et al.*, 2012; Arakawa and Erzurumlu, 2015). Whiskers are thought to play an important sensory role in newborn mammals, helping in nipple search and attachment, and guiding thermoregulatory huddling movements (Sullivan et al., 2003; Grant et al., 2012b). However, whisker development in species other than wallabies, rats and mice has not yet been described (Waite et al., 1991; Grant et al., 2012a), although it has been proposed that the whisker development schedule will vary across species and depend on their degree of maturity at birth (Grant and Goss, 2021).

1.2.3 Whiskers are associated with specialised brain areas

Many brain structures are associated with whisker touch sensing, such as the superior colliculus (Cohen and Castro-Alamancos, 2010; Gharaei et al., 2018), cerebellum (Bower, 1997; Bosman et al., 2010), striatum (Bosman *et al.*, 2011) and zona incerta (Bosman *et al.*, 2011; Evans *et al.*, 2019). Although the area that has excited the most interest is the Barrel cortex (Woolsey et al., 1975; Miller et al., 2001). This is an area in layer IV of the primary somatosensory cortex, and contains physical structures, which map topographically to the grid-like layout of the whiskers (Woolsey et al., 1975; Waite et al., 2006; Sawyer et al., 2015, 2016). This means that whisker signals from mechanoreceptors in the follicle can be traced from barrelettes in the brainstem to barreloids in the thalamus to barrels in the cortex (Woolsey et al., 1975; Waite et al., 2006; Sawyer et al., 2015, 2016). While the specialised topographic neural structures are present in some rodents (e.g. mice, rats, hamster (*Cricetus cricetus*); Woolsey *et al.*, 1975), marsupials (wallaby (*Macropus eugenii*); Woolsey *et al.*, 1975; Waite *et al.*, 2006), pinnipeds (California sea lion (*Zalophus californianus*); Sawyer *et al.*, 2015) and nocturnal primates (northern greater galago (*Otolemus garnettii*); Sawyer *et al.*, 2015), they are absent in species, such as the guinea pig (*Cavia porcellus*) and beaver (*Castor fiber*) (Woolsey *et al.*, 1975), and, in many species, we just do not know if they are present (Grant and Goss, 2021).

1.2.4 Whiskers were present in early mammals

Although whiskers are found in nearly all extant mammal species, they do not appear in fossil records, possibly due to the rare preservation of soft tissue in paleontological records. Therefore, pinpointing the time when whiskers emerged is problematic. It is even not yet known if whisker evolved independently from pelage hair, or not. However, comparative studies of extant mammalian species have proposed hypotheses. For instance, it has been suggested that whiskers are likely to have been present in early mammalian ancestors (Grant *et al.*, 2013), especially in the mystacial region in the therapsids, Theriodontia (Watson, 1931; Tatarinov, 1967; Findlay, 1968, 1970). Indeed, some authors have suggested that whiskers are phylogenetically older than pelage hair, and may have evolved directly from early ectodermal and mesodermal mechanoreceptors (Maderson, 1972; Chernova, 2006). In this case, they would have formed “sensory hairs” or proto-vibrissae first, which was then followed by mutations in the patterning genes causing the multiplication of the proto-vibrissae and next becoming pelage hair (or “proto-pelage”), which would play a role in protection of the skin from abrasion and enhancing thermoregulation in early mammals (Maderson, 1972, 2003). Since whisker muscle architecture is conserved in therian mammals (marsupials and placental mammals) (Grant et al., 2013a; Muchlinski et al., 2013), it is hypothesised that a common ancestor might have had mobile whiskers, more than 120 million years ago (Waite *et al.*, 1998; Mitchinson *et al.*, 2011; Grant *et al.*, 2013; Benoit *et al.*, 2016). Benoit *et al.* (2016) even proposed that functional facial whiskers were likely to present in non-mammaliaform Prozostrodontian therapsids, 240-246 million years ago. Subsequently, whiskers are likely to have played a role in early mammals and their ancestors, perhaps guiding locomotion in complex environments (e.g. densely vegetated and other obstacles) and in low-light conditions (e.g. nocturnality), as observed in extant mammals today.

1.2.5 Whiskers are functionally associated with ecology

Whiskers have been found to guide spatial orientation and locomotion in many small quadrupedal mammals, especially by guiding safe foot placements during walking, climbing, and gap-crossing (Sokolov and Kulikov, 1987; Brecht et al., 1997; Arkley et al., 2014, 2017; Grant et al., 2018). As tactile sensors, whiskers also play a role in object discrimination, such as to guide prey detection and identification in Australian water rats

(*Hydromys chrysogaster*) (Dehnhardt et al., 1999), rock hyrax (*Procavia capensis*) (Sarko *et al.*, 2015) and etruscan shrews (*Suncus etruscus*) (Anjum *et al.*, 2006), and even food manipulation (oripulation) in manatees (Marshall *et al.*, 1998; Reep *et al.*, 2001). Pinnipeds can even use their whiskers for hydrodynamic sensing, which is the detection of water movements, to follow prey items and conspecifics underwater (Miller, 1975; Dehnhardt et al., 1998, 2001, 2014; Gläser et al., 2011). Whiskers are also involved in social interactions and can be positioned to indicate aggression (Wolfe *et al.*, 2011). Whiskers also help to maintain contact between mother and pups, and between siblings in young animals (Sullivan et al., 2003; Grant et al., 2012b), and even between opponents during fighting (Ahl, 1986; Barnett, 2007).

Demonstrated throughout this overview is that whiskers vary in morphology and anatomy, as well as their degree of organisation and specialisation; therefore, there are species-specific differences. These are likely to be strongly associated with a particular species behaviour and ecology. Of terrestrial mammals, nocturnal, arboreal species have the longest and most numerous mystacial whiskers, which play a major role in guiding locomotion and environment exploration (Grant et al., 2018). Aquatic and semi-aquatic mammals, also have very specialised whiskers (Ginter et al., 2010, 2012; Dougill et al., 2020). Therefore, perhaps mammalian whiskers are particularly prominent in species, which forage and locomote around dark, complex environments – such as underwater, or through trees at night.

1.3 Aims and hypothesis based on mammalian whiskers form and function

Mammalian whiskers are well-known tactile sensors, whereas rictal bristles are only presumed to be tactile due to their similarity to whiskers both in terms of structure and position (Lucas and Stettenheim, 1972; Stettenheim, 2000), as well as the presence of mechanoreceptors around the rictal bristle follicles in some species (Küster, 1905; Cunningham, 2010). Such tactile function could enhance foraging behaviour, including facilitating obstacle avoidance in dense, complex environments (Küster, 1905; Lucas and Stettenheim, 1972) and orienting to prey items, as well as helping in the manipulation of captured prey (Cunningham et al., 2011). Indeed, if rictal bristles are similar to whiskers, then they may well guide navigation and foraging around dark, complex environments. As the function of the rictal bristles is fairly understudied and little is known about their anatomical structure (Cunningham et al., 2011), **the aim of this PhD thesis is to characterise avian rictal bristle form and function**.

In particular, it will:

i) describe bristle morphology and follicle anatomy
ii) describe the development of rictal bristles
iii) make predictions about rictal bristle evolution
iv) make predictions about bristle function
v) describe associated mechanosensory brain areas

This project will determine if rictal bristles are tactile sensors. It will adopt techniques in anatomy, morphology, behaviour and phylogenetic analyses. Using inspiration from the mammalian whisker literature, and assuming that rictal bristles might be similar to mammalian whisker, I hypothesise that:

i) Rictal bristle are associated with specialised follicle and neuroanatomical structures for touch
ii) Rictal bristles are important in developing chicks
iii) Rictal bristles may be present in a common avian ancestor
iv) Rictal bristles are associated with foraging in dark, complex environments.

This combination of chapters will demonstrate whether rictal bristles are tactile and describe their presence in birds.

Thesis overview

Chapter 2 will be a literature review. It will review previous literature on avian evolution, ecology, feather evolution and avian development. It will then describe avian sensory systems at the sensor and neural levels. It will describe sensory adaptations to nocturnality, and make relevant predictions of rictal bristle function.

In Chapter 3, rictal bristle morphology and follicle anatomy will be investigated in twelve species of Caprimulgiformes. It will compare the shape and length between Caprimulgiformes and describe the components present at the rictal bristles follicles, especially the presence/absence of mechanoreceptors in the immediate surroundings of their follicles. Finally, variation in rictal bristle morphology and follicle anatomy will be discussed in relation to species ecology.

Chapter 4 will identify the developmental schedule of feather development, rictal bristle emergence, and eye-opening in tawny frogmouth (*Podargus strigoides*) and barn owl (*Tyto alba*) chicks. It will also describe and compare the rictal bristle morphology and follicle anatomy in adults of both species, notably looking at the presence or absence of the mechanoreceptors around their follicles. This chapter will also investigate the tactile sensitivity of the rictal region during the development of chicks of both species, by stroking the rictal region and the control regions (top of the head, abdomen, proximal region) with a cotton bud. This will demonstrate the developing behavioural responses to rictal bristle touch, especially those associated with foraging behaviours, such as mouth movements and turning of the head.

Chapter 5 will record the presence, absence and morphology of the facial bristles in all birds and three selected clades, the Palaeognathae, the Caprimulgiformae, and the Passeriformes. It will investigate the evolution of facial bristles by doing bristle plesiomorphy reconstructions of stochastic traits, including the presence/absence of facial bristles, normalised rictal bristle length, and rictal bristle shape. This chapter will determine the ancestral character state of rictal bristle presence and morphology.

Chapter 6 will explore the association of rictal bristles in species with specific ecological traits, such as foraging and habitat type. It will determine which ecological traits are likely to predict the presence of rictal bristles in all avian species. A closer look at the Caprimulgiformae and the Passeriformes order will be conducted. The function of rictal bristles will be discussed by considering their ecology.

Chapter 7 will compare the brain shape and anatomy of the two species of nightjars, *Caprimulgus pectoralis* and *Caprimulgus vexillarius*, which both possess prominent rictal bristles. This chapter will especially look at specific mechanosensory related brain areas, such as the Wulst anatomy, and compare them between the two nightjars species, as well as other closely-related nocturnal species from Iwaniuk and Wylie (2006), including the spotted nightjar (*Eurostopodus argus*), the tawny frogmouth (*Podargus strigoides*), the oilbird (*Steatornis caripensis*), the feline owlet-nightjars (*Aegotheles cristatus*), the barn owl (*Tyto alba*), and boobook owl (*Ninox boobook*). Finally, it will look at the association between the rictal bristle presence and the volume of the brain, the Wulst and the optic tectum (supplied by Iwaniuk et al. (2008)) in 45 species (22 different families and 10 orders). Then, this chapter will discuss the association between brain anatomy, sensing and foraging traits.

Chapter 8 will, finally, discuss the findings and limitations of this work. It will also assess the remaining gaps of knowledge, and propose future recommendations.

Tinbergen's Framework

In order to investigate such a relatively understudied sensor, such as rictal bristles, a structure is helpful. Niko Tinbergen (Tinbergen, 1963) suggested that in order to obtain an integrative understanding of behaviour, four questions should be posed: How does it work? How does it develop? How did it evolve? And what is its function? Tinbergen's approach offers an established, comprehensive and logical framework (Tinbergen, 1963; Buchholz, 2007; Bateson and Laland, 2013) that takes into account the intertwined aspects of animal behaviour and ecology. Therefore, this thesis will follow Tinbergen's framework to provide the first comparative description of rictal bristle form and function. In particular, Tinbergen's first question *How do rictal bristles work?* will be addressed by chapter 3 (describing rictal bristle morphology and follicle anatomy) and chapter 5 (describing neural anatomy). *How do rictal bristles develop?* will be described in chapter 4. *How did rictal bristles evolve?* will be addressed in chapter 6. And *What is the function of rictal bristles?* will be addressed by chapter 7. These questions will be revisited in the thesis Discussion chapter (Chapter 8).

Chapter 2

Literature review

Avian evolution, development, sensory structures and adaptations

Chapter summary:

Throughout their evolution, birds have undergone a multitude of morphological and anatomical changes, resulting in a wide variety of species. Variations in morphological characteristics, breeding strategies, chick developmental schedules and sensory systems are all associated with each species ecological traits. Birds have notably outstanding anatomical adaptations in vision, hearing, smell and touch, suited to their specific ecological niches, as well as being associated with phylogeny, ecology and behaviour. Sensory systems are important, as they receive, relay and process specific information from the environment. The sense of touch is present throughout the animal kingdom, providing tactile information from inside and outside of the animal’s body, mediated by mechanoreceptors. Yet, due to its complexity, somatosensation is still under-studied and poorly understood, especially in nocturnal birds. So far, previous studies in bird tactile sensing have mainly been focused on bills, even though avian feathered skin also contains tactile sensors. Rictal bristles, for instance, are thought to carry out a tactile function that enhances foraging behaviour and facilitates obstacle avoidance, especially in dark, complex habitats. Therefore, rictal bristles could be acting as facial sensors in birds. This literature review will firstly provide an overview of avian evolution, development, behaviour, and brain morphology and anatomy. It will then describe avian sensory systems and associated species-specific ecology. Finally, sensory adaptations will be reviewed in relation to ecological and behavioural traits, specifically with nocturnality.

2.1 Overview of avian evolution and ecology

Our knowledge of avian evolution dates back to the Late Jurassic, with more than 160 million years (My) of discovered history (Mayr, 2016). From this period, almost all ancient bird species suffered extinction 65 My ago with only a small number of species passing through the extinction bottleneck of the K-Pg boundary (Feduccia, 2014). At least 22 lineages of avian species survived post-K-Pg, into the early Cenozoic, which have led to our modern birds (Fain and Houde, 2004; Feduccia, 2014).

Birds have undergone a multitude of morphological changes throughout their evolution, which has resulted in strong dissimilarities between modern birds and their closest relatives (Mayr, 2016). These changes have led to the evolution of many, morphologically-diverse avian species, with differences in body size, shape, and colour (Tietze, 2018). There are so far more than 10,000 contemporary avian species known (Gill, 2007). Contemporary avian species, or Neornithes, include two superorders: Palaeognathae, which is composed of the Ratites (Struthioniformes, Rheiformes, Apterygiformes) and the Tinamiformes (Figure 2-1), and the Neognathae (Galloanseres and Neoaves, Figure 2-1), which includes all the other avian orders (Fain and Houde, 2004; Gill et al., 2020). The Palaeognathae represent the most ancient lineage of species, within which all orders are flightless, with the exception of the Tinamiformes (Nomura and Izawa, 2017). Galloanseres contain two orders, the Galliformes (gamebirds) and the Anseriformes (Waterfowl). The Neoaves clade represents ~95% of all modern bird species but still has an unresolved basal polytomy (Hackett *et al.*, 2008). Neoaves are composed of seven supraordinal clades and three orphaned orders. The supraordinal clades are Strisores (nightjars, swifts, hummingbirds and allies; 1 in Figure 2-1), Otidimorphae (turacos, bustards and cuckoos; 2), Columbimorphae (mesites, sandgrouse and pigeons; 3), Mirandornithes (flamingos and grebes; 4), Eurypygimorphae (sunbittern, kagu, tropicbirds; 5), Aequornithes (core waterbirds; 6), and Telluraves (core landbirds; 7); the orphaned orders are Gruiformes (cranes and rails), Charadriiformes (shorebirds, gulls and alcids) and Opisthocomiformes (hoatzin) (Figure 2-1) (Suh, 2016; Gill et al., 2020).

Ecological traits differ between avian species and are associated with variation in morphological characteristics, such as foraging behaviour and bill characteristics (Nebel *et al.*, 2005). Variation in beak shape and size is generally known to be associated with specific feeding behaviours and cranial structures (Grant and Grant, 1993; Rico-Guevara et al., 2019). Niche competition has enabled birds to exploit diverse resources in terms of diet (aquatic predators, granivores, herbivores, frugivores, nectarivores, invertivores, vertivores, omnivores and scavengers), feeding tactics (dabbling, dipping, diving, plunge-diving, skimming, sallying, hawking, gleaning, scratching, scanning, probing and lunging), as well as different substrates (terrestrial, aquatic, and aerial) (Remsen and Robinson, 1990; Felice et al., 2019; Rico-Guevara et al., 2019). Likewise, niche competition has resulted in the evolution of nocturnal life histories that facilitates the exploitation of similar resources as diurnal species (Roots, 2006). Nocturnal birds have developed modifications in their sensory systems to adapt to low-light conditions, notably by enhancing or decreasing their reliance on vision, olfaction, hearing and tactile senses (Le Duc and Schöneberg, 2016). Therefore, contemporary bird species include a spectrum of diurnal, cathemeral (irregular activity pattern in which birds feeds during the day or night), crepuscular and nocturnal (obligate nocturnal) life-history traits (Figure 2-1) (Wu and Wang, 2019).

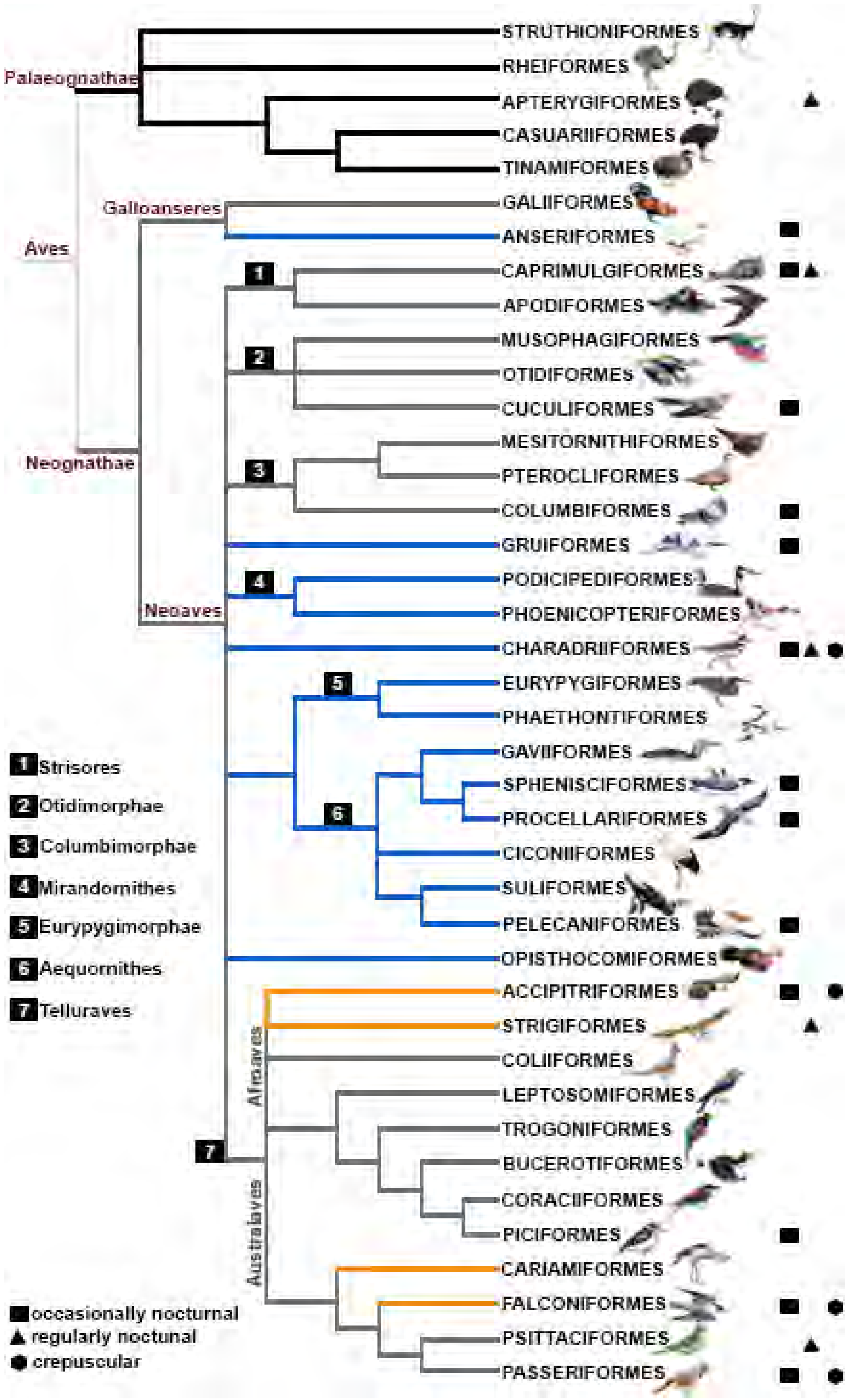


Figure 2-1. Phylogenetic tree of modern birds represented at a higher classification, i.e. based on the revised sequence of Orders (Gill *et al.*, 2020). Black coloured branches display the superorder of Palaeognathae while grey branches and other colours display the superorder of Neognathae. Following Wu and Wang, 2019, rectangle, triangle and hexagon symbols, respectively illustrate orders that are occasionally nocturnal, regularly nocturnal or crepuscular; species with no symbol juxtaposed are diurnals.

2.2 Overview of feathers

2.2.1 Evolution

Feather-like structures and feathers appeared in theropod dinosaurs and are thought to include two forms, a filamentous down-like feather (also called “dino-fuzz”) and a vaned feather form, similar to modern feathers (Gill, 2007). Feathers were thought originally to have evolved from scales but are more likely to have evolved as novel epidermal structures, developing from the selection of a follicle, which would grow a tubular appendage (Prum and Brush, 2002). Feather evolution may have followed a five-stage model (Figure 2-2) (Prum and Brush, 2002; Gill, 2007). The first evolutionary mechanism would be the origin of an undifferentiated collar producing an unbranched, hollow, tubular feather (Stage I, Figure 2-2), followed by the formation of barb ridges resulting in a mature feather with tufts of unbranched barbs (vanes) attached to a calamus (hollow base) (Stage II, Figure 2-2). Next, helical displacement of the forming barb ridges results in a simple bipinnate feather developing with barbs, branched with barbules, fused to a central rachis (Stage III, Figure 2-2). Barbules would then differentiate forming the first closed, vaned pennaceous feather (Stage IV, Figure 2-2). Finally, the diversification of barbule structure and position would lead to the formation of feathers with aerodynamic functions, i.e. closed asymmetrical vaned feathers, and other feather structure, such as after-feathers (Stage V, Figure 2-2). Feathers then evolved in different forms, shapes, sizes and textures, and constitute the most complex appendage that appeared in bird evolution (Chuong et al., 2000; Stettenheim, 2000; Prum and Brush, 2002; Yu et al., 2004).

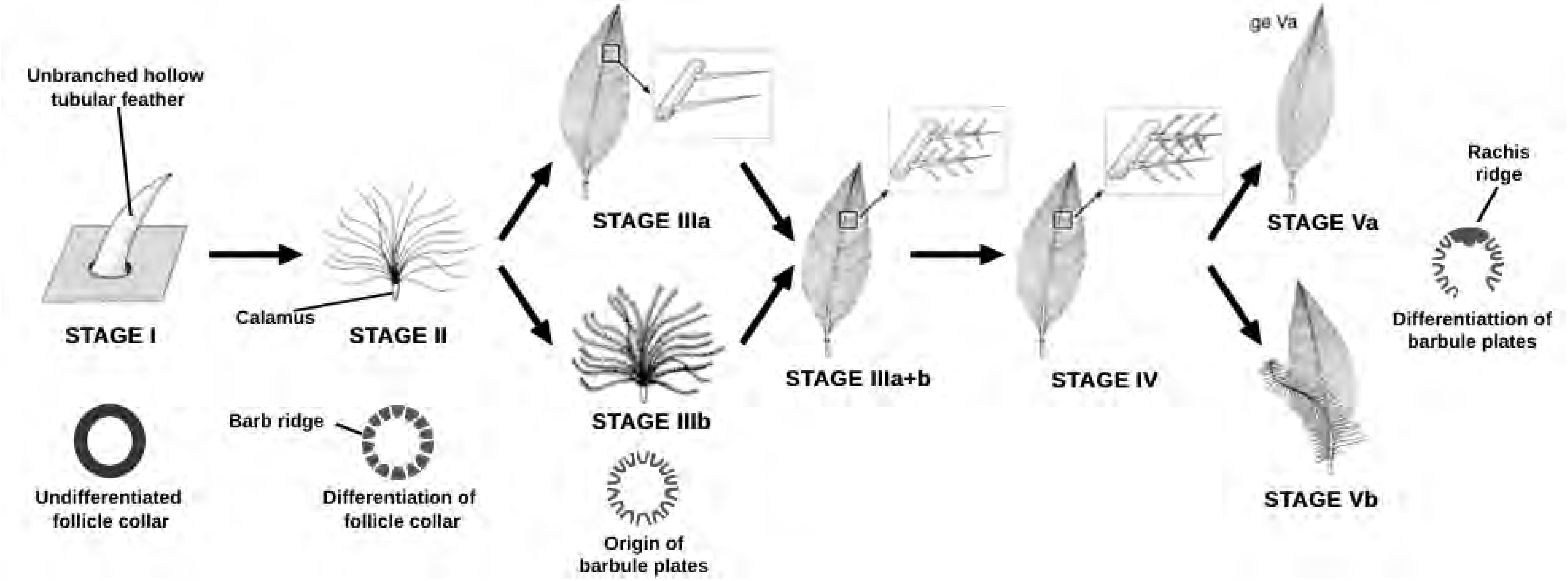


Figure 2-2. Diagram illustrating the hypothesised evolutionary model of the origin of feathers and their diversification (Prum and Brush, 2002). Stage I represents the first feather as a hollow cylinder generated by an undifferentiated tubular collar. Stage II represents a mature feather with a tuft of unbranched barbs attached to a calamus, originated by the barb ridge differentiation of the collar. Stage III displays the helical displacement of the forming barb ridges, resulting in the formation of a bipinnate feather with barbs fused to a central rachis, and barbules. Stage IV displays the first closed, pennaceous vaned feather thanks to the differentiation of barbule structures (e.g. a hook at their tip). Stage V portrays a closed asymmetrical vaned feather (Va; resembling modern remiges and rectrices) results of a lateral displacement of the new barb locus, and an afterfeather with a single calamus, which originates from the division and lateral displacement of the new barb loci.

2.2.2 Feather morphology

Feathers are composed of a central shaft, whereby the calamus is anchored in a dermal follicle, and the main external part, the rachis support the barbs when branched (Figure 2-3A) (Gill, 2007). Birds have five main types of feather: contour feathers, down feathers, filoplumes, semiplumes, and bristles. The filoplumes are fine hair-like feather with long rachis and tuft of barbs at the tip (Figure 2-3A; Pass, 1989). Semiplumes have an entirely plumulaceous vane and a long rachis (Figure 2-3A; Pass, 1989). Down feathers are small, soft, plumulaceous feathers that have their rachis shorter than their barbs (Figure 2-3A, Table 2-1) (Pass, 1989). In addition, there are three types of down feathers: the natal downs that are present at, or soon after, hatching in some species, and the body downs that occur in various parts of the body, with species-specific distribution patterns (Figure 2-3A; Pass, 1989), and the powder downs, which are present all over the body or in clusters of some certain species, such as herons and pigeons (Table 2-1) (Podulka et al., 2004). Contour feathers are the most abundant type of feather; these are well-developed plumulaceous and pennaceous feathers that cover the entire body, neck and limbs: the coverts (the small feathers on the wing, tail and occasionally around the ears) and the well-developed flight feathers, or remiges (wing feather) and the rectrices (tail feathers) (Figure 2-3A, Table 2-1) (Pass, 1989; Stettenheim, 2000; Yu et al., 2004). Finally, the bristles are fairly stiff, tapered, hair-like feathers that sometimes have barbs at the base of their rachis (Figure 2-3A; Table 2-1) (Pass, 1989), with branched bristles called semi-bristles (Gill, 2007). Bristles are mainly facial and occur on the upper rictal (above the rictus of the mouth), narial (nostrils) (Figure 2-3B), lower rictal (beneath the rictus), lorial (between the bill and eye), and interramal(beneath the beak) regions (Figure 2-3C, Table 2-1), as well as on the forehead and eyebrow areas (Stettenheim, 1973; Cunningham et al., 2011).

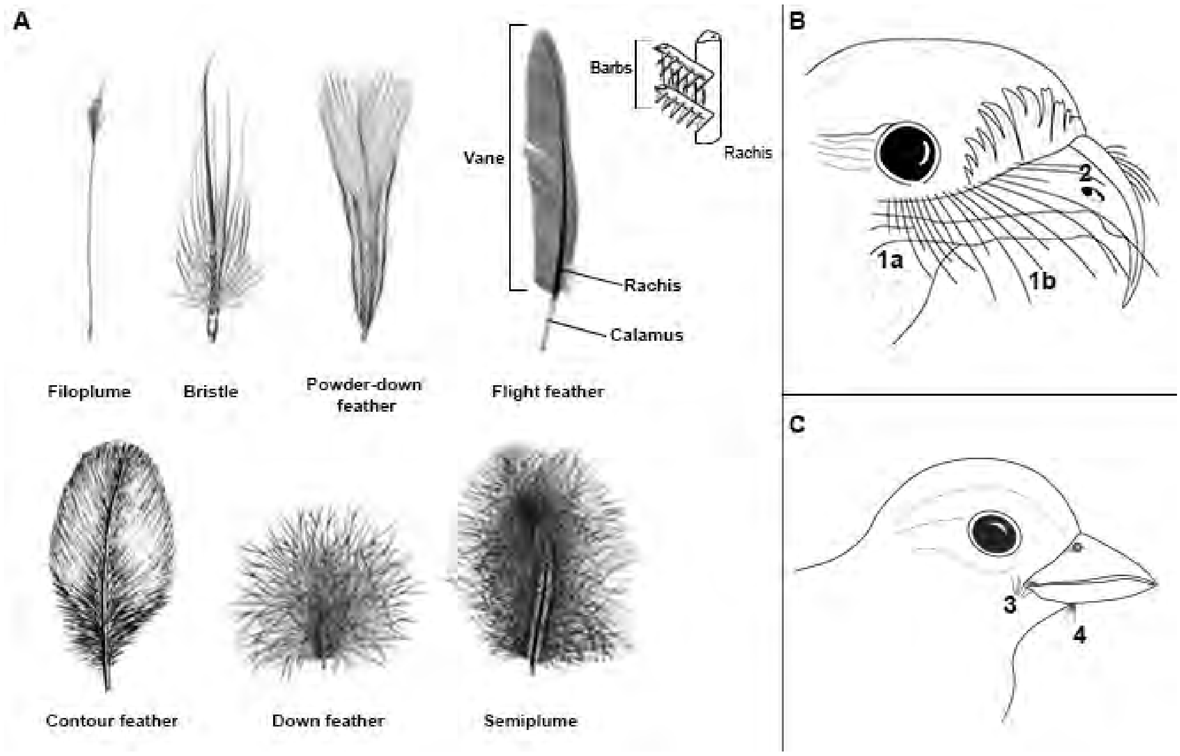


Figure 2-3. A) Different types of feathers: filoplume, bristle, powder-down feather, contour feather, down feather and semiplume (Source: Nett and Tully, 2003). **B and C)** Schematic drawing of the oilbird face and a passerine bird face, respectively, illustrating the position of different bristle types: 1) Rictal region including 1a upper rictal bristles and 1b lorial bristles, 2) Narial bristles, 3) Lower rictal bristles and 4) Interramal bristles.

2.2.3 Feather anatomy

Like hair, feathers are produced and supported by dermal follicles and are surrounded by muscle fibres, except for filoplume follicles (Pass, 1989; Stettenheim, 2000). Feathers are arranged in clusters or tracts (Clench, 1970), of which the distribution (termed pterylosis) varies between species (Olivera-martinez et al., 2003; Fliniaux et al., 2004; Ho et al., 2019). Smooth muscle fibres form bundles that connect follicles together and attach them to the skin matrix by elastic tendons ramifying into collagen (Stettenheim, 2000). The size and arrangement of these muscles fibres depend on the type of feather, its size and function (Stettenheim, 2000). For example, the flight feather displays more muscle fibres at their follicles to adjust the feather position (Stettenheim, 2000). There is also fat in the dermis and subcutis that forms a hydrostatic cushion for the feathers (Stettenheim, 2000). The dermis also contains Herbst corpuscles that are positioned close to the outer wall of the feather follicles. Their position allows them to collect information on feather position and pressure received during flight (Stettenheim, 2000). Filoplumes have more Herbst corpuscles around their follicles than the contour feathers that typically only have 1 or 2 Herbst corpuscles (Stettenheim, 2000).

2.2.4 Feather function

Feathers are complex integumentary appendages specialised primarily for flight, providing insulation, contributing to aerodynamics and coloured for communication and camouflage (Stettenheim, 1972; Gill, 2007). They also play a functional role in swimming, water repellency, water transport, protection, support, cleanliness, sound production, hearing and tactile sensation (Stettenheim, 2000; Gill, 2007). Contour feathers cover the body surface providing insulation, reducing air turbulence in flight or friction while swimming (Table 2-1). The primary function of flight feathers is associated with aerodynamics, especially the remiges (wing), with the rectrices (tail) involved in steering and braking during flight, as well as courtship display or sound production (Table 2-1) (Gill, 2007). Down feathers provide a high-quality insulation, which is especially important in small birds living in cold climate and birds that swim, dive or float on water (Table 2-1) (Podulka et al., 2004). Natal downs emerge from the same follicle that will later produce the contour feather, covering the body and aiding body temperature maintenance (Table 2-1) (Podulka et al., 2004). Body downs are also present in adult birds. They are produced by their own follicle and distributed throughout the plumage, under the contour feathers, and provide thermal insulation (Table 2-1) (Podulka et al., 2004). Body downs are especially abundant in species living and foraging in water (Podulka et al., 2004). Powder-down feathers are specialised feathers, modified from down feathers, present in specific areas amongst body downs and contour feathers (Figure 2-3A) (Pass, 1989). They do not molt but grow continuously, disintegrating at their tips to produce a fine powder, consisting of keratin granules, that serve as a preening and waterproofing agent (Table 2-1) (Pass, 1989; Stettenheim, 2000; Yu et al., 2004). They are not present in all species, occurring predominantly in herons, bitterns, cockatoos and sandpipers amongst other species (Pass, 1989; Stettenheim, 2000).Semiplumes aid in insulation, aerodynamics and can serve as a courtship ornament too (Table 2-1) (Stettenheim, 2000; Gill, 2007). Filoplumes are deemed to possess a proprioceptive function and relay information about the orientation of neighbouring feathers, helping to adjust the position of flight feathers (Stettenheim, 2000), and possibly help in monitoring airspeed (Table 2-1) (Gill, 2007). Finally, facial bristles and semi-bristles are thought to have a protective and sensory function, and may provide a tactile function (Table 2-1). Rictal bristles have been suggested to be a tactile organ analogous to mammalian vibrissae (Seneviratne and Jones, 2008). Indeed, avian feathers are diverse. There are many different types, which vary in form and are specialised to their specific functions. A summary of these can be seen in Table 2-1.

Table 2-1. Summary on the form and function of the different types of feathers.

Feather		Function	Form
Type	Subtype		
Contour feathers	Contour feather	Insulation, reduction of air turbulence while flying or swimming. Appearance.	Pennaceous vane with plumulaceous portion, and a rachis longer than the longest barbs
	Flight feather	Aerodynamics (remiges), Steering and braking up flight (rectrices), courtship display, sound production	
Down feathers	Natal down	Insulation in chicks	Soft, plumulaceous feather typically lacking a rachis or having a rachis shorter than the longest barbs
	Body down	Insulation in adults	
	Powder down	Insulation, waterproofing and preening	
Semiplumes		Insulation, aerodynamics, courtship ornament	Entirely plumulaceous vane and a long rachis
Filoplumes		Proprioception, position adjustment of flight feathers, airspeed monitoring	Hair-like feathers having a rachis with few or no barbs
Bristles	Semi-bristles	Protective sensory function	Fairly stiff, tapered, hair-like feathers that sometimes have barbs at the base of their rachis
	Facial bristles	Sensory function (possible tactile function?)	

2.3 Overview of the avian brain and neural sensory structures

Despite a last common tetrapod ancestor, presumably from the Carboniferous period more than 330 Mya, avian and mammalian brains have evolved in parallel and developed analogous structures (Burish *et al.*, 2004; Walsh and Milner, 2011). In mammals, the encephalisation of the brain is recognised to have evolved with the olfactory bulbs, indicating an increase of the dependence on olfactory perception (Rowe *et al.*, 2011). Avian brain size and shape is diverse (Walsh and Milner, 2011), and brain enlargement is associated with visual, auditory and somatosensory systems (Nomura and Izawa, 2017).

The avian brain is divided into three main partitions: the forebrain, the brainstem and the spinal cord. Each region and their associated function will be defined below.

2.3.1 Forebrain

The forebrain plays a large role in the processing of sensory information, cognition, and memory (Walsh and Milner, 2011). Two major divisions make up the forebrain, the telencephalon (or cerebrum) and the diencephalon.

Telencephalon

The telencephalon (Figure 2-6) is divided into two main areas, the dorsal part (or pallium), and the basal part or (subpallium) (Jarvis, 2009a). The subpallium or basal ganglia is subdivided into two distinct domains: pallidum, containing the globus pallidus (GP) and the ventral pallidum (VP), and the striatum (Figure 2-6A) (Reiner et al., 2004; Jarvis et al., 2005). The pallium, however, subdivides in four domains: the hyperpallium, mesopallium, nidopallium and arcopallium (Figure 2-6B) (Reiner *et al.*, 2004; Reiner *et al.*, 2005; Jarvis *et al.*, 2005). The pallium also includes the hippocampal complex, a pair of olfactory bulbs and the piriform cortex (Reiner *et al.*, 2005).

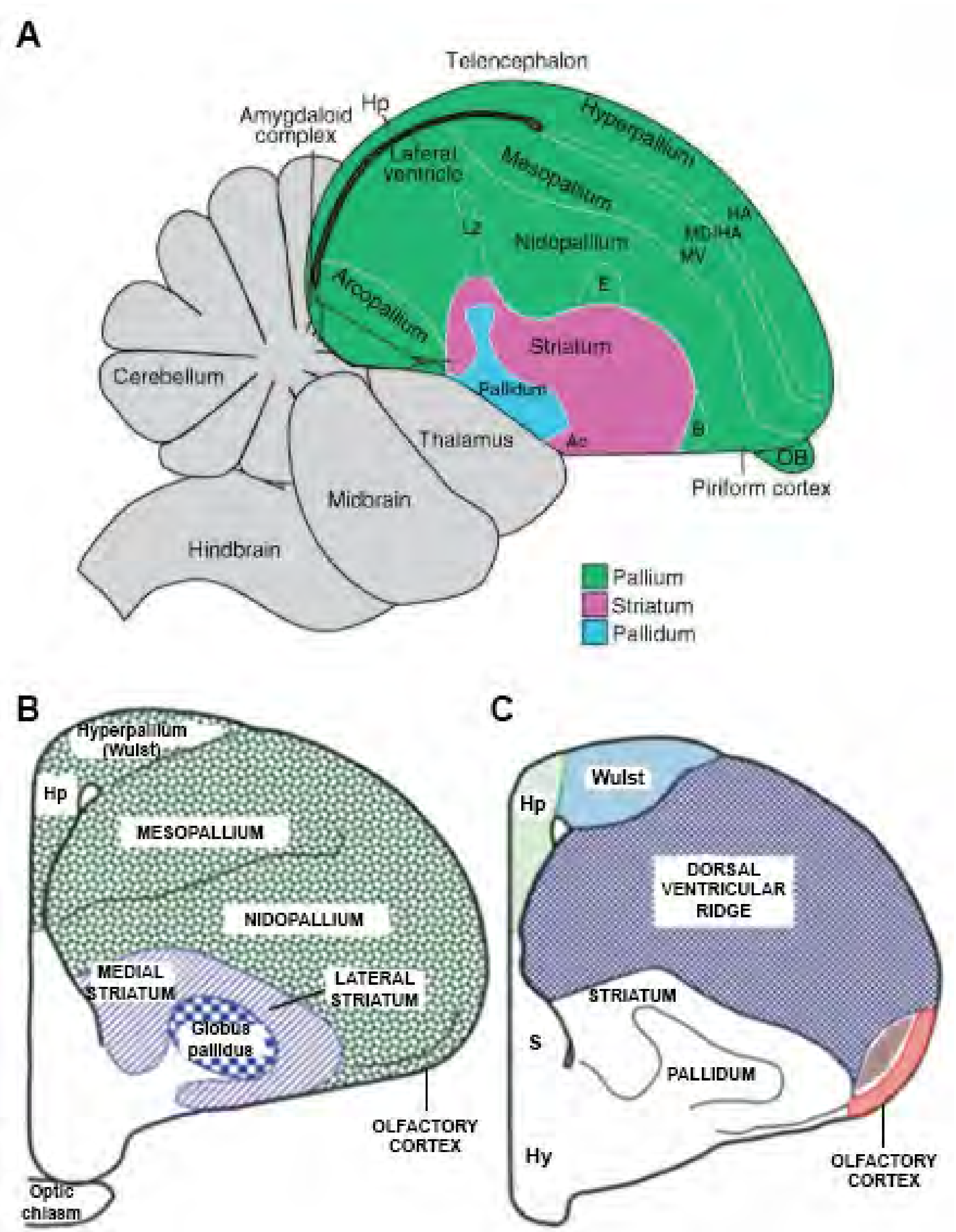


Figure 2-6. A) Organisation of the adult telencephalon of birds. Illustration of the sagittal view of the brain from (Jarvis, 2009b) with a colour code for the Pallium, Striatum and Pallidum domains. Abbreviations: Ac accumbens; B basorostralis; Cd caudate; E entopallium; GP globus pallidus, internal (i) and external (e) segments; IHA interstitial hyperpallium apicale; HA hyperpallium apicale; Hp hippocampus; L2 field L2; MD dorsal mesopallium; MV ventral mesopallium; OB olfactory bulb; Pt putamen. **B)** Schematic line drawing of mid-telencephalon transverse brain section of the pigeon from Reiner *et al.* (2005). The schematic shows the current interpretation of the telencephalic organization with the current interpretation and nomenclature (Reiner *et al.*, 2004). **C)** Schematic of the major areas present in the telencephalon of pigeon brain (transverse section) from Reiner *et al.* (2005). Abbreviations: Hp, hippocampus; S, septum.

Together with the nidopallium, the hyperpallium plays a role in sensory information processing due to specific nuclei receiving direct visual, auditory, and somatosensory inputs from the thalamus. The arcopallium, nidopallium and hyperpallium also all play a role in motor control (Figure 2-6) (Reiner *et al.*, 2005). The hyperpallium, is composed of three different parts, the hyperpallium apicale (HA), intercalatum (HI) and densocellulare (HD) (Reiner *et al.*, 2005). The HA receives extraencephalic and intraencephalic projections from the Wulst, as well as somatosensory inputs from the lemniscal pathway (thalamus) (Medina and Reiner, 2000; Reiner et al., 2004). The nidopallium comprises of the nidopallium caudolateral (NCL), the higher vocal centre (HVC) and three main areas including Field L, the entopallium (E) and the nucleus basorostralis pallii (Bas), which receive extraencephalic information. Both Field L and the entopallium receive auditory inputs from the nucleus ovoidalis (Ov) and visual input from the nucleus rotundus (Rt) of the thalamus, respectively (Figure 2-8) (Karten *et al.*, 1973; Reiner *et al.*, 2004, 2005).

Mesopallium, nidopallium and arcopallium together form a region of nuclear grey matter called the dorsal ventricular ridge (DVR) and the semi-layered hyperpallium forms the Wulst region (Figure 2-6C). Both regions are thought to be unique to birds (Jarvis et al., 2005; Reiner et al., 2005) and are associated with avian cognition (Jarvis et al., 2005; Güntürkün and Bugnyar, 2016). The Wulst region (Figure 2-6C) forms a distinct prominence named the eminentia sagittalis delimited from the rest of the telencephalon by a furrow called the vallecula (Walsh and Milner, 2011). This is present in all modern bird species; but varies in shape, size and position within species (Walsh and Milner, 2011). The relative size of the Wulst appears to be associated with improved vision, eye orbit orientation and an enhancement of depth perception associated with the evolution of stereopsis (Medina and Abellán, 2009; Walsh and Milner, 2011; Iwaniuk and Wylie, 2020). Hence, an especially enlarged Wulst is found in the Strigiformes (Medina and Abellán, 2009; Walsh and Milner, 2011). The Wulst is divided into two regions: the larger part is situated more caudally than the other and is associated with the primary visual area; the second smaller part extends to the rostral pole of the brain and is associated with the somatosensory system (Delius and Bennetto, 1972; Karten et al., 1973; Funke, 1989; Wild and Williams, 2000). The visual part of the Wulst comprises neurons that relay cues like orientation, movement direction, spatial frequency and binocular disparity (Iwaniuk and Wylie, 2006). Altogether, the bimodal neurons of the Wulst are layered in different sheets of neurons called lamina and have different degrees of lamination occurring within species (Iwaniuk and Wylie, 2006).

More lamination in the Wulst is associated with more complex topographical maps in the brain (Iwaniuk and Wylie, 2006). For this reason, gradation differences of the Wulst between taxa could reflect functional differences (Iwaniuk and Wylie, 2006). In addition, an interaction between visual and somatic inputs also seems to exist in the Wulst, leading to the suggestion that the Wulst might be involved in coordinating visual-limb activity notably in the forelimb (Deng and Wang, 1993).

Diencephalon

The diencephalon contains structures like the thalamus and hypothalamus. The avian thalamus is highly differentiated and contains a large number of thalamic nuclei arranged in groups (Powell and Kruger, 1960; Powell and Cowan, 1961; Goodman and Schein, 1974). The central inferior group contains the nucleus ovoidalis (Ov) and nucleus rotundus (Rt), which are specific to the ascending auditory pathway and the tectofugal pathway, which is involved in projecting visual information to the entopallium in the telencephalon (Figure 2-7) (Goodman and Schein, 1974; Reiner et al., 2004; Wylie et al., 2009). The central superior group contains the principal optic nucleus that relays the thalamofugal visual pathway from the retina to the caudal, visual Wulst (Goodman and Schein, 1974; Wylie et al., 2009), while the somatosensory-specific thalamic nucleus dorsalis intermedius ventralis anterior (DIVA) projects to the rostral Wulst (Figure 2-7) (Funke, 1989) The thalamic nucleus basalis (Bas), on the other hand, receives trigeminal input directly from the principal sensory trigeminal nucleus (PrV) of the thalamus (Figure 2-7) (Reiner *et al.*, 2004), which receives somatosensory information from the bill tip through the maxillary and mandibulary branches of the trigeminal nerve (Iwaniuk and Wylie, 2020). Therefore, the size of the Bas, along with PrV, vary amongst species and are notably enlarged in bill-probing tactile foragers, such as shorebirds, woodcock, waterfowl and kiwi (Cunningham et al., 2013; Iwaniuk and Wylie, 2020).

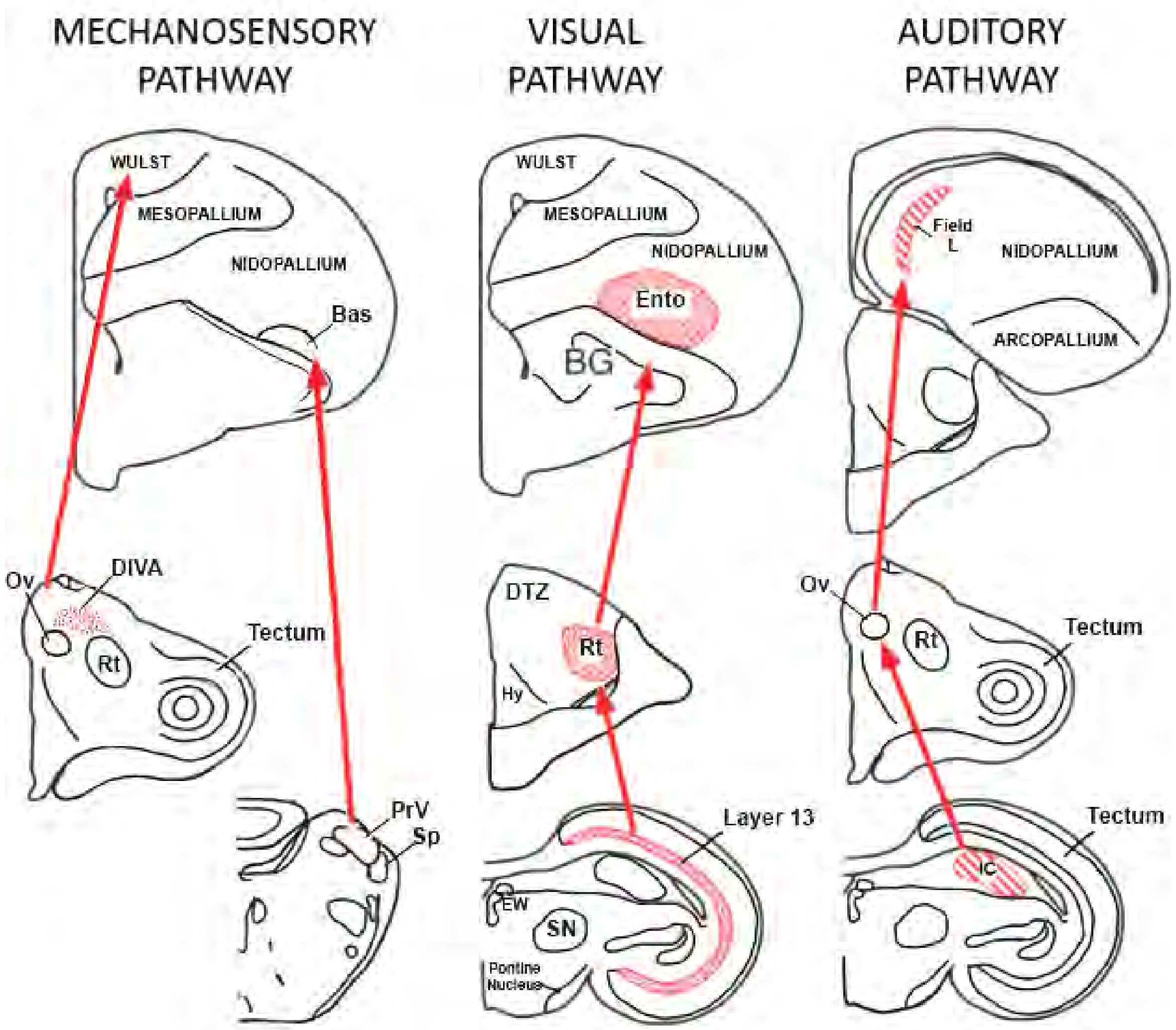


Figure 2-7. Schematic diagrams of frontal sections through the cerebrum, the diencephalon, and the midbrain of the pigeon. The diagrams illustrate the different cell groups involved in avian mechanosensory, visual and auditory pathways. Abbreviations: Bas, nucleus basalis; BG, basal ganglia; DIVA, nucleus dorsalis intermedius ventralis anterior; Ento, entopallium; EW, nucleus of Edinger-Westphal; Hy, hypothalamus; IC, inferior colliculus; Ov, nucleus ovoidalis; PrV, principal sensory nucleus of the trigeminal nerve; Rt, nucleus rotundus; SN, substantia nigra; Sp, nucleus subprincipalis. Modified from Reiner et al, 2005.

2.3.2 Olfactory lobes

The olfactory bulbs (OB), similarly to the Wulst, is situated at the rostral part of the telencephalon (Figure 2-6), vary in size between species and likewise, an enlargement of the OB goes hand in hand with a more elaborate sense, the sense of smell (Walsh and Milner, 2011). For instance, seabirds and vultures have large OBs, and so have a more developed sense of smell, and kiwi species, which are known to have an even more enlarged OB, have consequently the most developed sense of smell in birds (Figure 2-8) Bang and Cobb, 1968; Walsh and Milner, 2011). In contrast, most water birds (Figure 2-8), marsh dwellers, and waders have an intermediary OB size, which suggests a useful olfactory sense while other species that have a relatively small OB possibly could rely a less on their sense of smell (Bang and Cobb, 1968).

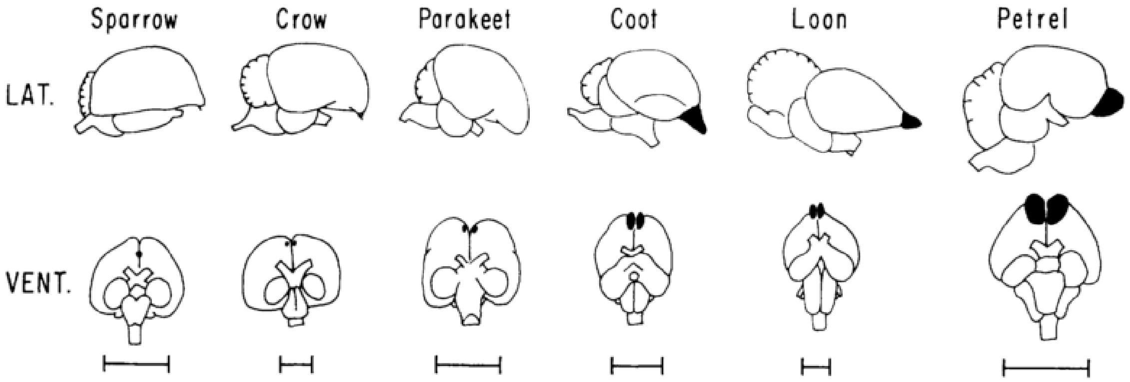


Figure 2-8. Olfactory bulbs morphology in six avian families: sparrow (passerine), parakeet, crow, coot (water bird), loon (diver water bird), petrel (seabird). (Bang and Cobb, 1968).

2.3.3 Brainstem

The brainstem is composed of the mesencephalon (or midbrain) and the rhombencephalon (or hindbrain) (Figure 2-6) (Jarvis *et al.*, 2005; Walsh and Milner, 2011).

Mesencephalon

The mesencephalon contains the peduncles of the forebrain and optic tectum involved in the motor reflex pathway for the oculomotor nerves (Walsh and Milner, 2011). The tegmentum part of the mesencephalon is involved in broad motor and oculomotor control while the tectum part processes visual and auditory inputs relayed by the diencephalon (Figure 2-8) (Walsh and Milner, 2011). The optic tectum is an elaborately laminated structure, composed of 15 layers (Figure 2-9) (Ramon y Cajal, 1911; Wylie et al., 2009)) that is associated with developed optic lobes (Figure 2-9) (Goodman and Schein, 1974), and primarily involved in visual processing (Reiner and Karten, 1982). Layer 13, or the striatum griseum central, is composed of large multipolar neurons that project into the thalamic nucleus rotundus (Rt), which then project into the entopallium of the telencephalon (Reiner and Karten, 1982; Wylie et al., 2009; Fernández et al., 2020). These regions are involved in processing visual information, such as brightness, colour, pattern discrimination, simple motion and looming stimuli (Iwaniuk *et al.*, 2010).

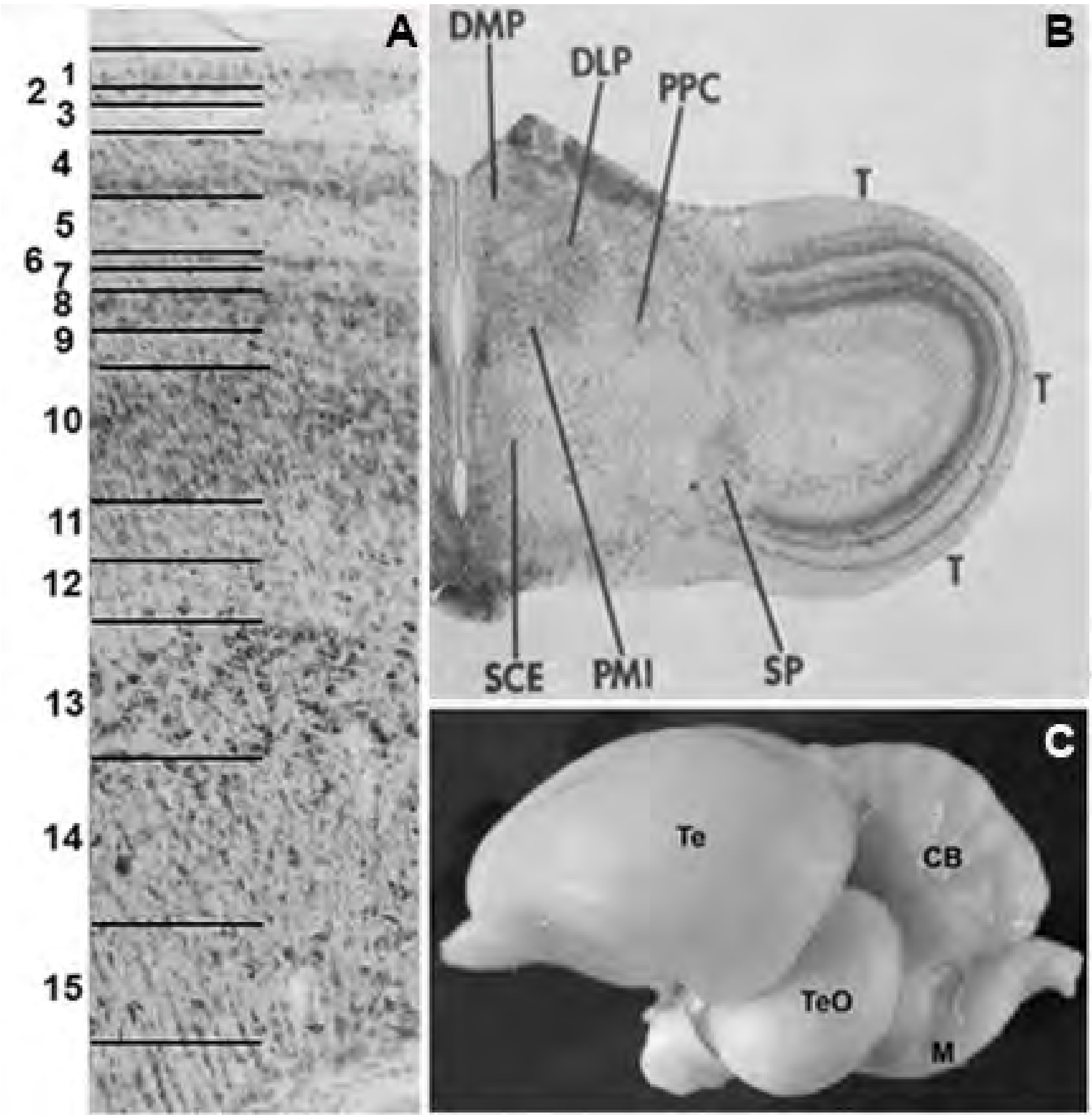


Figure 2-9. Avian optic tectum (TeO): **A)** Photomicrograph through the optic tectum of the pigeon, illustrating the 15 layers described by Ramon y Cajal (1911). **B)** Transverse section of the pigeon mesencephalon illustrating the well-laminated avian optic tectum (Nissl staining). **C)** Lateral view of the pigeon brain showing the conspicuous size of the TeO. Abbreviations: DLP, nucleus dorsolateralis posterior thalami; DMP, nucleus dorsomedialis posterior thalami; PMI, nucleus paramedianus internus thalami; PPC, nucleus principalis precommissuralis; SCE, striatum cellular externum; SP, nucleus subprectectalis; T, optic tectum. (Modified figures of Goodman and Schein, 1974 and Wylie *et al.*, 2009).

Rhombencephalon

The rhombencephalon (Figure 2-6) is principally involved in motor control and rhythmicity, more precisely in the control of heartbeats, respiration, and digestion via autonomic centres and seven cranial nerves (CN) exiting from rhombencephalon (numbered caudally from V to XII) (Walsh and Milner, 2011). The rhombencephalon is composed of the metencephalon containing structures such as the pons and cerebellum, and the myelencephalon (or medulla oblongata) (Goodman and Schein, 1974). The cerebellum is formed of eleven primary folia of which the degree of foliation, as well as its overall size and morphology, vary considerably from a vertebrate to another (Iwaniuk and Hurd, 2005; Walsh and Milner, 2011). The diversity of cerebellum foliation and morphology is phylogenetically dependent and associated with behaviour and function. For instance, the folium III is known to receive tactile stimuli from the tail and leg and so is involved in hindlimb function (Iwaniuk et al., 2005), whereas folia VII and VIII are involved in the visual and auditory system (Whitlock, 1952; Gross, 1970; Clarke, 1974).

2.3.4 Cerebrotypes

Sensory pathways are associated with functional significance and enlargement of one pathway often accompanies a diminution of another (Wylie et al., 2015). Consequently, sensory trade-offs play an important role in the evolution of sensory systems and, by extension, in the evolution of the brain. However, the composition of the brain is influenced by many environmental constraints and selective pressures related to a species life-history (Iwaniuk and Hurd, 2005). Similarities in life-history traits or behaviours can drive the evolution of cerebrotypes, which are specific types of brains shared by many species (Iwaniuk and Hurd, 2005). Indeed, with the evolution of the avian brain, specific cerebrotypes have evolved convergently in disparate taxa (Iwaniuk and Hurd, 2005). Specific ecological niches, as well as phylogeny, may also contribute to the brain organisation (Iwaniuk and Hurd, 2005; Buchanan et al., 2013; Wylie et al., 2015). Hence, the need to place the study of brain evolution and brain-behaviour relationship in a phylogenetic context (Wylie et al., 2015).

The cerebrotypes of a given species represent volume fractions of all components of the brain referring to a function (Burish *et al.*, 2004). Consequently, each cerebrotype volume varies depending on brain function (Iwaniuk and Hurd, 2005; Walsh and Milner, 2011). The relative size of the regions indicates the importance played by the function in the life of the animal (Kawabe *et al.*, 2015). Therefore, cerebrotypes relate primarily to behaviour and ecology, with some phylogenetic constraints (Iwaniuk and Hurd, 2005). For example, the dichotomy between altricial and precocial mode of hatchling development itself seems to influence the volume of the brain (Iwaniuk and Nelson, 2003) and the size of brain regions (e.g. the forebrain area by the telencephalon) (Walsh and Milner, 2011). The longer the period of development the bigger the relative brain volume. Therefore brain volume is influenced by the duration of the fledgling incubation period and post-fledging care period (Iwaniuk and Nelson, 2003). Species with similar locomotor behaviour, such as terns, swifts and hummingbirds, also have similar cerebrotypes, i.e. large cerebella compared to the rest of the brain (Iwaniuk and Hurd, 2005). However, the strongest evidence of niche-specific cerebrotypes is found in species with similar foraging methods, especially prey capture (Iwaniuk and Hurd, 2005). Prey capture strategies that rely on vision to guide the hunt has resulted in the convergent evolution of similar visual fields and eye movements (Wallman and Pettigrew, 1985; Moroney and Pettigrew, 1987). Therefore, species such as kingfishers and raptors, which are both visually-guided predators hunting small vertebrates and large invertebrates, are likely to have a visual system of similar relative size and share almost identical relative nidopallial volumes (nidopallium being a major site of sensory information integration) (Iwaniuk and Hurd, 2005). Another foraging strategy giving rise to similar cerebrotypes is nocturnal hunting from a perch, such as frogmouths and owls (Iwaniuk and Hurd, 2005). Indeed, frogmouths and owls, both possess binocular vision (Pettigrew, 1986), similar saccadic eye movement (Wallman and Pettigrew, 1985) and are solitary species that do not engage in complex social behaviour. They, therefore, share similarities in the morphology of the cerebellum and the enlargement of their telencephalon, which is mostly due to the enlargement of the Wulst (Iwaniuk and Hurd, 2005). In contrast, in parrots and passerines, the enlargement of the telencephalon is due to the expansion of the three other telencephalic parts - the nidopallium, the mesopallium and striatopallial complex, which are involved in problem solving, tool use and social behaviour (Scharff and Nottebohm, 1991; Barber et al., 1999; Timmermans et al., 2000; Ball and Balthazart, 2001; Lefebvre et al., 2002; Plummer and Striedter, 2002). Therefore, the enlargement of the telencephalon of parrots and passerines may be more considered more of a “cognitive” cerebrotype, associated with complex cognitive functions rather ecological specialisations (Iwaniuk and Hurd, 2005).

2.4 Overview of avian development

2.4.1 Chick development

At hatching, the functional maturity of behaviour, physiology and anatomy of the chicks vary across species linked to the level of parental care (precocial, super-precocial and altricial developmental types) and the environment (Starck and Ricklefs, 1998). Therefore, variation amongst taxa of postnatal development features produces a broad spectrum of attributes, which has been used by ornithologists to separate birds into altricial-precocial of development types (Table 2-2) (Starck and Ricklefs, 1998). In altricial species, chicks hatch in an underdeveloped state (without downy plumage and eyes closed) and remain in the nest while growing, requiring care and feeding from their parents for a long period (Table 2-2). Whereas in super-precocial and precocial species, chicks hatch in an advanced state (indicated by fully developed feathers and downy plumage, respectively), have their eyes open, are capable of feeding by themselves, and require little (precocial) to no (super-precocial) parental care (Table 2-2) (Starck and Ricklefs, 1998; Gill, 2007). Most precocial species tend to be ground nesting and chicks are ready to leave the nest soon after hatching whereas most altricial species tend to nest above ground and chicks grow in the nest before being able to leave the nest on their own (Bicudo et al., 2010).

Table 2-2. Descriptive characters used to diagnose the developmental types in bird hatchlings on the altricial-precocial spectrum (Starck and Ricklefs, 1998).

Spectrum	**Plumage**	**Eyes**	**Nest attendance**	Parental care
Superprecocial	Contour feathers	Open	Leave	Protection only or None
Precocial-2	Natal down			Brooding
Precocial-3				Parental food-guiding
Precocial-4				Parental feeding
Semiprecocial			Remain in the area	
Semialtricial-1			Stay	
Semialtricial-2		Closed		
Altricial	None			

2.4.2 Plumage development

The difference between altricial and precocial hatchling plumage is thought to be associated with heat transfer (conferred by parents, siblings) and heat conservation (Starck and Ricklefs, 1998; Bicudo et al., 2010). The duration of the presence of natal downs and their abundance varies between development types: natal downs are more abundant and are kept longer in precocial species to help them maintain their body temperature out of the nest, whereas altricial species depend on the warmth provided by brooding parents, so nestlings tend to have a more sparse natal downs for more efficient heat transfer and they tend to keep the downy plumage for a shorter time (Podulka et al., 2004).

Feathers follicles and the pattern of the feather tracts are formed at the embryonic stage and persist throughout the birds' life (Lucas and Stettenheim, 1972). Natal downs emerge from the same follicle as the contour feathers, therefore, natal downs are often still weakly attached to the tip of the subsequent growing contour feather, which give the feathery unkempt appearance of some young juveniles (Podulka et al., 2004). Chicks go through different plumage development stages before fledging: hatchling plumage consisting in bare skin to the entire body covered of down feathers (contour feathers in superprecocial); natal downs are then superseded by juvenile feathers similar in structure to adult feathers, which in turn are replaced by the adult plumage (Bellairs and Osmond, 2005). The production of feathers demands energy, and during its development, 21 to 40% of the chicks energy budget is spent on its growth, maintenance, temperature regulation, activity and excretion (Gill, 2007).

2.4.3 Chick behaviour and sensory development

Avian embryonic developmental differences are mainly controlled by genetics. Egg size, shape, pigmentation and incubation period vary across species, therefore influencing embryonic growth rates (Bellairs and Osmond, 2005; Gill, 2007). Incubation periods can be as short as 10 days for some species (e.g. woodpeckers, cuckoos, small songbirds) or as long as 80 - 90 days for others (e.g. albatrosses, kiwis) (Gill, 2007). Sensory abilities can vary during the development schedule of a species. For instance, although mechanoreceptors (tactile corpuscles; 2.5.4) develop during the last third of embryonic development of the chicks (Saxod, 1988) and are fully developed before hatching in precocial species, in altricial species the development of tactile corpuscles only starts at birth (Páč and Malinovský, 1988). Altricial nestling sensory and motor abilities are not advanced and develop from hatching. Most altricial species are not able to make begging calls after hatching, but they rapidly develop these calls, which become louder and more persistent as the nestling grows (Podulka et al., 2004). Begging calls are often accompanied with exaggerated body movements, both made to solicit their parents to make provisioning efforts (Leonard and Horn, 2001). Once the parents bring food, they solicit the nestlings to open their beak to deliver food, the nestling gaping mouth aids food delivery as well as serving as a visual stimulus, attracting the parents' attention (Gill, 2007). Their eyes open one fourth of way into the nestling stage, which allow them then to orient their gape to the source of the stimuli (e.g. parents, human hand or forceps holding food) and allow them to detect potential danger (Podulka et al., 2004). Soon after, the nestlings are able to grasp objects with their feet and able to locomote better, adopting different position (crouching when defensive, posterior raising when defecating) (Podulka et al., 2004). Precocial chicks do still require time to adjust to their surroundings (Podulka et al., 2004). For instance, although having their eyes open from hatching, it takes a few hours before chicks start reacting to light stimuli and within a day the chicks are able to regulate their body temperature (Podulka et al., 2004). Chicks also rapidly start to proceed upwards, locomote and begin to feed themselves (e.g. Australian brush-turkey chicks (*Alectura lathami*)) (Podulka et al., 2004).

2.5 Sensory adaptations

Sensory adaptations promote a gain in sensitivity performance and resolution. Hence, appropriate sensor reception requires sensory organs that are dedicated to specific stimuli (Catania and Henry, 2006). Birds are no different and have adaptations in vision, hearing, smell and touch. Nocturnality is one adaptation that has led to both sensory, and neural, specialisations. Partial or complete scotopic adaptation appears in distinct taxa and has multiple evolutionary events (Braun and Huddleston, 2009). Therefore, depending on the niche they have adapted to, birds have enhanced and softened their photosensitivity and further developed at least one other sensory system than vision in order to perceive their surroundings or support behaviours such as foraging, mating, predation (Le Duc and Schöneberg, 2016).

2.5.1 Visual system

Environmental variation and selection are expected to modulate visual organs, morphologically and anatomically (Yokoyama, 2000; Garamszegi et al., 2002) to promptly detect environmental signals (Yokoyama, 2000; Osorio and Vorobyev, 2008). Birds seem to largely rely on vision, probably more so than other vertebrates (Iwaniuk *et al.*, 2010).

Eye size

Birds rely on visual information to navigate environments with precision and speed, in order to feed or dive in dim-light at depth foraging for fish (Lisney et al., 2015; Martin, 2015). Vision also helps to discriminate colourful sexual characters (Yokoyama, 2000;

Martin and Osorio, 2010; Lisney et al., 2015). As a result, different bird species have developed different visual photosensitivity and resolution according to their ecology and behaviour (Martin and Osorio, 2010). The variation of light levels during the day or different environments cause modulation of the eye aperture to adjust retinal illuminance. The perceived retinal illuminance is based on the diameter of the pupil, which is controlled by the iris (Martin and Osorio, 2010). Retinal illumination is species-specific, the dynamic range of light levels received by the eye depends on their environmental or behavioural needs (Martin and Osorio, 2010). For instance, birds such as king penguins (*Aptenodytes patagonicus*) have adapted to cope with daylight and dim-light at depth: they dilate their pupil to a large circular aperture when diving or in scotopic environments, and constrict their pupil to a square-shaped pinhole when they are at the surface in full day light (Martin, 1999). Furthermore, larger eye sizes, associated with larger brain areas, allow birds to process more visual information and capture many environmental cues (Symonds *et al.*, 2014). Larger eyes may allow birds to have more photoreceptive cells and therefore perceive more light, as well as benefiting from a higher spatial resolution (Garamszegi *et al.*, 2002). The enlargement of the eye means that birds become more sensitive to “disability glare” (Ho and Bilton, 1986). Enlarged eyes have evolved to gather more light from the surrounding, but they also catch the glaring light of the sun coming directly from above, causing diffusion of light inside the eye chamber (Ho and Bilton, 1986). This phenomenon seems to have a bigger impact in species with larger eyes and can affect prey detection, especially when prey are perceived in a low contrast environment (Le Claire and Rabinovitch, 1982). Larger eyes are usually associated with an external structure that plays a protective role against the “disability glare”, such as eyelashes and enlarged eye brows (Martin, 2007).

Visual fields

Surrounding visual information is received by both eyes and forms a three-dimensional image determined by the angle created by optic axes centred on the head (Martin and Osorio, 2010). This three-dimensional space is described as the visual field, which is species-specific and highly dependent on feeding behaviour and parental provisioning for the young (Martin et al., 2005; Martin and Osorio, 2010). Visual fields guide the beak or feet towards food items and chicks’ gape (Martin, 2007). Three main types of visual field topography of the frontal binocular region occur amongst bird species (Type 1, 2 or 3;

Figure 2-4), defined by the position of the bill and eyes (Martin and Osorio, 2010). Binocular vision is the result of the two eyes looking at the same position in space (Martin, 2007).

The majority of bird species orient their bill tip to find food sources or prey, and to find the beak of their young (Martin, 2007; Martin and Osorio, 2010). However, they do not necessarily see the tip of their bill, but rather the items held in their bill (Martin, 2007). These species possess a Type 1 visual field (Figure 2-4), meaning that the projection of their bill falls centrally or immediately below the centre, and form a narrow field of the frontal binocular region (20-30º maximum), which is associated with a blind area (from 40º to 100º in width) (Martin, 2007; Martin and Osorio, 2010). Although the binocular field is frontally narrow, it is vertically long and varies between species (Martin, 2007). For instance, herons possess a binocular field that extends to 180º vertically and 20º horizontally. It seems that such arrangement allows the bird to see its feet while standing motionless, head at the horizontal, waiting for prey items (Martin, 2007).

Alternatively, species vulnerable to predation by living in open habitat have a Type 2 visual field, and the hatchlings of these species are precocial (Figure 2-4) (Martin, 2007; Martin and Piersma, 2009). Type 2 species require vision for both vigilance and foraging, which demands a trade-off between binocular and panoramic vision (Martin and Piersma, 2009). Type 2 visual field is described by a frontal binocular field ≤10º wide to the direction of the bill, while 180º of sagittal binocular vision also provides the bird with vision above and around the head (Martin, 2007).

Type 3 species include owls, who have a large blind area above the head that can attain a maximum width of 160º at the rear (Figure 2-4) (Martin, 2007; Martin and Osorio, 2010). Their broad frontal binocular vision extends to 50º wide with the projection of the bill tip falling just outside the lower periphery (Martin, 2007; Martin and Osorio, 2010). Type 3 has been suggested to be associated with nocturnality, however, it is not entirely known which ecological or behavioural factors are linked with the extensive binocular overlap (Tansley, 1965; Martin, 2007). Nocturnal species, such as some herons, or nightjars and the oilbird (*Steatornis caripensis*) phylogenetically close to owls, do not possess a Type 3 visual field but instead possess a Type 1 (Figure 2-4) (Martin, 2007).

Some species lack binocularity and move their eyes instead (Martin, 2007). Eye movements result in a reduction in the width of the blind area behind the head and possibly increase the resolution of a specific visual area, enhancing acuity in prey detection (Martin, 2007). Avian eyes do not have to rotate together in the same direction, but can be dissociated and moved independently; this ability increases the variation of visual field configurations, but only a few species are capable of eye movements, including herons (Ardeidae family), Great Cormorants (*Phalacrocorax carbo)* and hornbills (Bucerotidae family) (Martin and Coetzee, 2004; Martin, 2007).

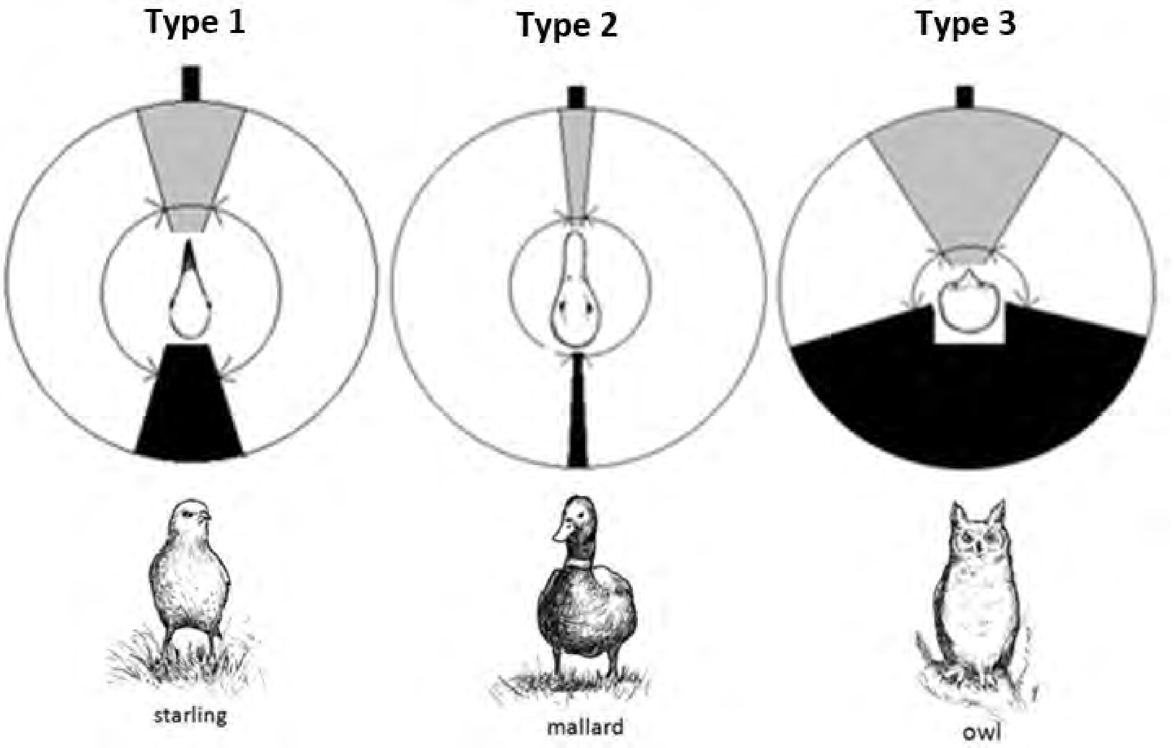


Figure 2-4. Schematic diagram of the different types of the visual field in birds. Respectively, the grey and the black areas represent the binocular overlap and the blind area. The regions in between the two latter areas (no filling colour) represent two monocular fields forming the visual areas of the bird. For each visual type, an example of species possessing the visual field is juxtaposed to the diagram: a drawing of a starling (*Sturnus* spp.) for Type 1, a drawing of mallard (*Anas platyrhynchos*) for Type 2, and a drawing of an owl (Strigiformes) for Type 3 (Source: http://estebanfj.bio.purdue.edu/birdvision/visualfields.html).

Adaption to nocturnality

Avian nocturnal species have larger eyes than avian crepuscular and diurnal species, with a higher sensitivity to light than spatial resolution (Corfield *et al.*, 2011). The overall size and shape of the eye plays a role in optimising retinal illuminance (Hall and Ross, 2007). Nocturnality is associated with larger corneal diameters and larger axial lengths than diurnal and cathemeral avian species (Hall and Ross, 2007). Diurnal birds have larger axial lengths relative to their corneal diameters, while crepuscular and cathemeral species present intermediary ratios (Hall and Ross, 2007). Most nocturnal species have their visual system reinforced and adapted to night vision (Hall and Ross, 2007; Martin and Osorio, 2010). For instance, larger pupils and a shorter focal length give a wider-angle and helps maximize light sensitivity at night. A bigger aperture of the eye is also more common in nocturnal birds (Land and Nilsson, 2012). As for the visual acuity, nocturnal species have a higher concentration of rod receptors (Walsh and Milner, 2011), and together with increased photoreceptors specialised in scotopic vision and an enlargement of the eye, provides nocturnal birds with bigger effective photoreceptive areas than those present in diurnal species (Walls, 1942; Martin, 1999; Land and Nilsson, 2012).

2.5.2 Auditory system

Acoustic canal

Most bird species have an external acoustic meatus (or ear-canal) that goes from the outer ear to the middle ear, and which is located approximately halfway between the posterior end of the skull and the gape of the bill, below the line of the eye (Saiff, 1974, 1976). The auditory canal contains a specialised feather distributed around the ear opening (Saiff, 1974; Walsh and Milner, 2011). Birds benefit from these "acoustic" feathers as they protect the hearing organs from air turbulences during flight and form a structure through which sound waves are channelled to the brain, helping in prey detection and prey localisation (Gill, 2007). This system is particularly efficient in Strigiformes as it allows the bird to locate the prey on sound alone (Walsh and Milner, 2011). However, not all birds possess an external auditory system; Casuariiformes (e.g. emu and cassowary), Struthioniformes (ostriches) and Falconiformes (Falcons) have a naked external acoustic meatus (Walsh and Milner, 2011). Sound waves are transmitted from the ear canal to the eardrum (tympanic membrane), where they produce vibrations in the pressure-sensitive fluid system of the inner ear, encoding sounds into information that are mediated to the nervous system by the ear ossicle, the columella (Beason, 2004; Gill, 2007). The inner ear has two functions, equilibrium and hearing. The latter takes places in the cochlea, which length is species-specific (Beason, 2004) and is capable of sound detection and sound acquisition with the help of basilar papilla (Fay and Popper, 2000). Indeed, the auditory sensory epithelium, that is the basilar papilla, vary in length across species, therefore determining the frequency range to which a given species is sensitive, and the number of hair cells that varies accordingly (Gleich and Langemann, 2011).

Adaptation to nocturnality

Nocturnal owls, such as the barn owl (*Tyto alba*), have an enhanced hearing abilities. They can locate prey in complete darkness due to their specific ear funnel and the bilateral asymmetry of their external ear openings on the skull (Gill, 2007). Owls have large ear flaps, anteriorly and posteriorly located, which help adjust the size of their ear opening and enhancing their acoustic acuity (Schwartzkopff, 1973; Gill, 2007). The asymmetrical position of the ear on each side of the owl skull helps to locate prey faster and with more precision (Knudsen and Konishi, 1979; Gill, 2007). Some avian species like swiftlets (*Aerodramus sp.* and *Collocalia sp.*) and oilbirds (*Steatornis caripensis*) use echolocation to navigate their way through dark caves (Gill, 2007; Brinkløv et al., 2013) by emitting sonar clicks match their most sensitive frequencies of hearing (Knudsen and Konishi, 1979).

2.5.3 Olfaction

Olfactory system

Olfaction plays a major role in foraging (locating food and navigation) and reproduction (Walsh and Milner, 2011; Corfield et al., 2014). The avian olfactory system has olfactory receptors (ORs) that are composed of both cilia and microvilli in olfactory receptor cells, whereas across other vertebrates they have either ciliated (i.e. marsupials, turtles, lamprey) or microvillar cells (i.e. ratfishes, sharks and skates) (Eisthen, 1997). These olfactory receptors allow birds to sense any airborne chemical stimuli or odours, such as pheromones, offspring, predators as well as territories and food, which makes olfaction a crucial sensory system for fitness and survival (Khan *et al.*, 2015). ORs are contained in the mucosal olfactory epithelium of the third nasal chamber of birds and are connected to olfactory nerves that will relay the information in the olfactory lobes (telencephalon) (Walsh and Milner, 2011; Corfield et al., 2014).

Adaption to nocturnality

Nocturnal and crepuscular birds often have a more developed sense of smell than diurnal birds and tend to have larger olfactory-bulbs (Healy and Guilford, 1990). Nocturnal ground-dwelling kiwis (*Apteryx sp.*) have limited visual capabilities, but compensate by having enhanced olfaction (Cunningham et al., 2013). Kiwi species have large olfactory bulbs and olfactory chamber, reflecting a highly developed sense of smell, which helps them to locate food over short distances (Walsh and Milner, 2011; Corfield et al., 2014). The relative size of the olfactory bulbs is not only associated with nocturnal and crepuscular activity, but also with foraging, diet, navigation, nesting strategy and habitat (Cobb, 1960; Healy and Guilford, 1990). For example, Leach's storm petrel (*Oceanodroma leucorhoa*) can detect their nest burrows in the dark using smell, rather than relying on visual or auditory cues (Grubb Jr, 1974).

2.5.4 Somatosensation

The sense of touch, or somatosensation, comprises the detection of stimuli from the surface and inside the body. Somatosensation involves two tactile sensing abilities: proprioception which is the sense of position and movement, and haptic perception, which is touch and includes vibration and pressure (Prescott *et al.*, 2016). Within the animal kingdom, all species with a central nervous system (CNS) are capable of sensing a tactile stimulation mediated by mechanoreceptors (Prescott *et al.*, 2016). The sense of touch in birds is relatively understudied and has mainly focussed on the bill (Gentle and Breward, 1986; Piersma et al., 1998; Le Duc and Schöneberg, 2016). However, somatosensation is well developed in birds with a complex network of sensory fibres covering their whole bodies (somatic and visceral), which are terminated by specialised cutaneous cells or mechanoreceptors, the tactile corpuscles (Saxod, 1988; Roots, 2006; Gill, 2007).

Mechanoreceptors

The avian skin has the same basic outer epidermis and inner dermis structure as mammals, possessing somatosensory receptors (Pass, 1989). Tactile corpuscles are situated in the dermis, in the immediate feather follicle surroundings and the bill skin, while the epidermis only contains free nerve endings (Saxod, 1988). Bird skin contains different types of mechanoreceptors: slowly adapting mechanoreceptors, i.e. Ruffini endings, as well as rapidly adapting mechanoreceptors that include the sensory corpuscles, Herbst, Gandy and transient Merkel cells receptors (Malinovský, 1988; Necker, 2000). Tactile corpuscles are composed of a dendritic zone (receptor) of pseudounipolar neurons (type of sensory neurons of which the axon split into two branches, one running to the periphery, the other to the spinal cord), Schwann cells and a capsule (Malinovský, 1988). Their distribution and number vary within taxa, linked to their feeding behaviour (Stettenheim, 2000; Cunningham et al., 2013). Ruffini endings or corpuscles are the encapsulated modification of free stretch receptors (Figure 2-5A) (Necker, 2000). Although well known in mammals, Ruffini corpuscles have only been identified in bird bill and joint capsules, and to date, there is no morphological evidence of them being found in feathered skin (Necker, 2000). Merkel cell receptors are located in the dermis and are composed of single or multiple cells, and can even be organised in a similar fashion to Grandry corpuscles (Figure 2-5B and C) (Necker, 2000). They have been mainly found in the bill and tongue of birds, as well as identified in the toe and feathered skin, and their presence is speculated in the inner ear as secondary sensory cells (Necker, 2000). Grandry corpuscles are composed of encapsulated, stacked Grandry cells (2 or more) with discoid axon endings in between the cells (Figure 2-5D) (Necker, 2000). They are dermal vibrotactile sensors that detect velocity, especially present in the bill of aquatics birds (Necker, 2000; Stettenheim, 2000; Cunningham et al., 2013). Herbst corpuscles are the largest, most elaborate and the most distributed tactile corpuscles in the avian skin (Necker, 2000; Stettenheim, 2000; Gill, 2007; Cunningham et al., 2013). Indeed, Herbst corpuscles are ellipsoidal, lamellar, multi-layered corpuscles that are highly sensitive to pressure and rapid mechanical deformations such as vibration (Figure 2-5E) (Gottschaldt, 1985; Necker, 2000; Stettenheim, 2000; Cunningham et al., 2013). Herbst corpuscles are found in the deep dermis of legs and feathered skin, as well as being present in abundance the sensitive bill tips of aquatic birds, such as Anseriformes and sandpipers (Scolopacidae) (Gottschaldt, 1985; Necker, 2000; Stettenheim, 2000; Cunningham et al., 2013).

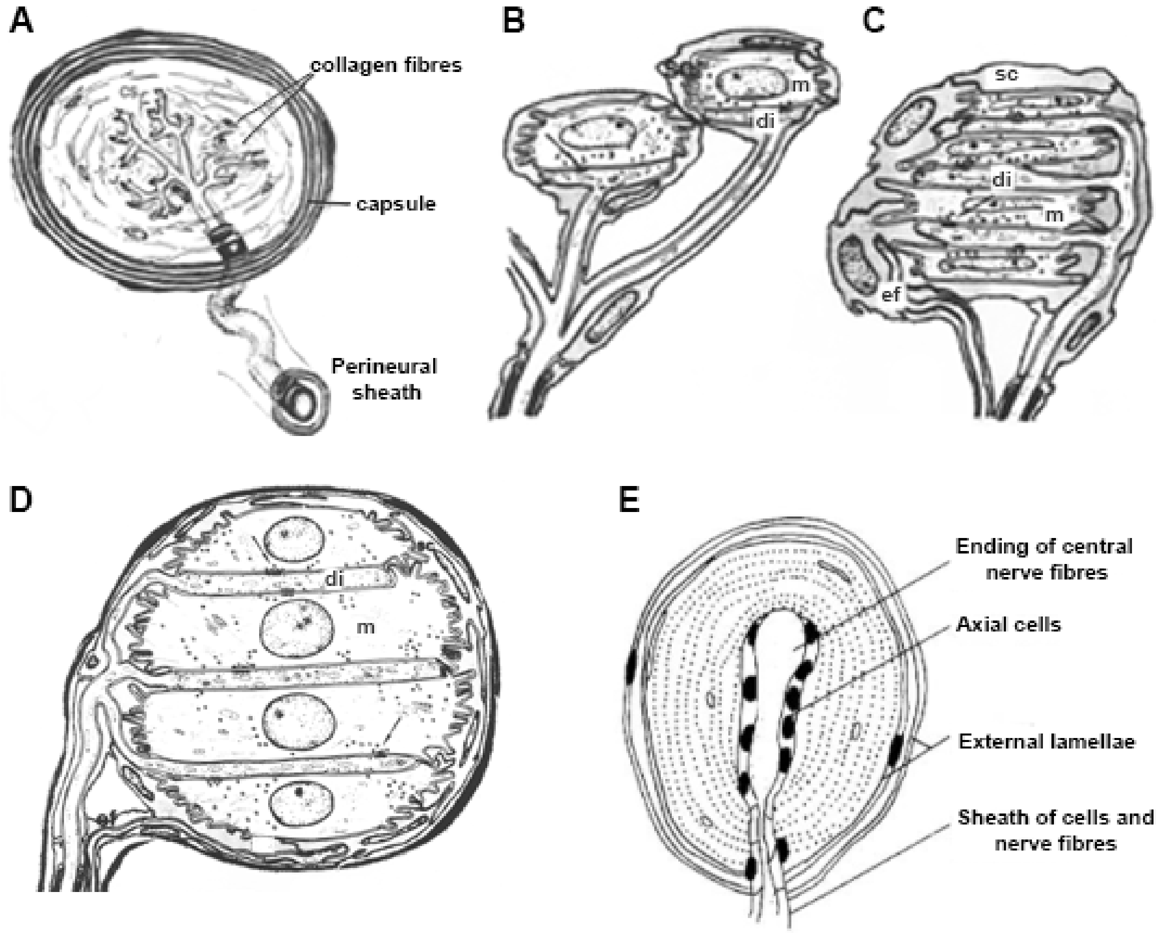


Figure 2-5. Different types of mechanoreceptors: A) Ruffini endings/corpuscle, B) Merkel cell receptor, C) Merkel cell corpuscle, D) Grandry corpuscle, E) Herbst corpuscle. Abbreviations: di, disk-like afferent nerve endings; ef, efferent fibre; m, Merkel cell; sc, Schwann cell. Modified figures from Necker (2000) and Gill (2007).

Sensitive bill

Avian feeding ecology often depends upon a trade-off between vision and touch sensory systems (Cunningham et al., 2013; Corfield et al., 2015). Some birds rely more on vision (e.g. eagles, the oilbird (*Steartornis caripensis*)) while others rely more on their specific tactile system to catch food (Corfield *et al.*, 2015). Beaks, especially the tomia (the edges of the beak), are one of the most diverse traits in birds (Stettenheim, 2000) and contain complex sensory structures in order to carry out these strategies (Cunningham et al., 2013). Certain families of birds, such as Scolopacidae (shorebirds), Threskiornithidae (ibises) and Apterygidae (kiwis), possess a tactile sensory beak specialisation named "remote touch" (Gerritsen and Meiboom, 1985; Piersma et al., 1998; Cunningham et al., 2007, 2010). In Ibises, this specialisation is present in species living in habitats ranging from terrestrial grasslands and forest to open water (e.g. lakes and lagoons) (Cunningham et al., 2010). This vibrotactile sensory mechanism, mediated by numerous pits in the bone of the bill-tip, contains clusters of mechanoreceptors (Gerritsen and Meiboom, 1985; Piersma et al., 1998; Stettenheim, 2000). Two types of mechanoreceptors are present in these sensory pits: Herbst corpuscles and Grandry corpuscles (Piersma et al., 1998; Stettenheim, 2000; Walsh and Milner, 2011; Cunningham et al., 2013; Le Duc and Schöneberg, 2016). These mechanoreceptors are likely to be involved in remote touch sensing, harvesting sensory cues from the ground (Cunningham et al., 2013). Herbst corpuscles specifically detect seismic (vibrational) signals from invertebrates moving in the ground and pressure disturbances of sessile prey (Gerritsen and Meiboom, 1985; Piersma et al., 1998). These tactile sensory cues are then relayed to the telencephalon and received as somatosensory projections in the rostral Wulst (I.3.2.1.2; Iwaniuk and Wylie, 2006; Cunningham *et al.*, 2013). The bill tip of kiwis and shorebirds is sensitive to vibration, which allows them to spot the location of invertebrate in the ground, using that "remote-touch" sensing (Gerritsen and Meiboom, 1985; Piersma et al., 1998; Cunningham et al., 2007). In ibises that are more aquatic than terrestrial, the bill tip is more densely and extensively packed with sensory pits, most likely helping in locating prey both within the water column and underground (Gerritsen and Meiboom, 1985; Piersma et al., 1998; Cunningham et al., 2010). Similarly, spoonbills, which are exclusively aquatic foragers, exhibit a high number of sensory pits in their bill covering more than 50% of its length (Swennen and Yu, 2004). Terrestrially foraging kiwis have a relatively low number of sensory pits in their bill (~300), which covers about 12.5% of its length (Cunningham et al., 2007). This suggests that remote touch sensory mechanisms are species-specific and associated with foraging habitats and behaviour. It is especially common in probe-foraging birds (Cunningham et al., 2010).

Sensitive feathers

The dermis contains Herbst corpuscles that are positioned close to the outer wall of the feather follicles where they may sense pressure and vibration when the feather is moved by its muscle fibres or an external force (Lucas and Stettenheim, 1972). Their position would, therefore, allow them to collect somatosensory information, notably on feather position and pressure received during flight (Stettenheim, 2000). For instance, filoplumes have more Herbst corpuscles around their follicles than the contour feather, which only have 1 or 2 Herbst corpuscles as they are deemed to possess a proprioceptive function and relay information about the orientation of the neighbouring contour feathers (Stettenheim, 2000). Herbst corpuscles can also be found beside almost every contour feathers on the front of the head but are rarer in the surroundings of rectrices, and dorsal cervical, femoral and alar regions (Lucas and Stettenheim, 1972). Moreover, they have been identified around the base of facial bristles follicles in owls (Küster, 1905). Hence, rictal bristles have been suggested to be a tactile organ analogous to mammalian vibrissae (Seneviratne and Jones, 2008).

Adaptations to nocturnality

Shorebirds are mainly diurnal but commonly migrate and forage at night (Mouritsen, 1993). Shorebirds, such as dunlins (*Calidris alpina*), use visual cues paired with remote touch sensing behaviour during daytime, whereas in the darkness they rely heavily on their sensitive bill to detect food (Mouritsen, 1993). Certain species, such as the kiwis and species belonging to the Caprimulgiform order also possess prominent rictal bristles, which are thought to carry out a tactile function (Keast and Saunders, 1991; Cunningham et al., 2011). For instance, the nocturnal oilbird has long rictal bristles and an enlarged Wulst, which may receive more somatosensory than visual inputs, as it seems to have lost its stereopsis abilities as a result of living deep in caves (Iwaniuk and Wylie, 2006). Some Caprimulgiformes such as frogmouths and owlet-nightjars possess stereopsis abilities, whereas other species such as potoos and nightjars do not (Iwaniuk and Wylie, 2006). Therefore, the reliance on tactile cues might depend on species-specific ecology (e.g. activity pattern).

2.6 Rictal bristles: the avian facial sensor?

2.6.1 Are rictal bristles tactile?

Rictal bristles are commonly seen around the beak of both nocturnal and diurnal birds (Figure 2-3) (Lederer, 1972; Persons and Currie, 2015). Rictal bristle morphology varies greatly between species and are notably present in Apterygidae (kiwis), Caprimulgidae (nightjars), Aegothelidae (bushtits), Accipitridae (eagles), Mimidae (mockinbirds) and Tyrannidae (tyrant flycatchers), Libiidae (barbets) and Strigidae (owls) (Ripley, 1945; Lederer, 1972). The precise function of these bristles is still unknown. Initially, the presence of rictal bristles was thought to be related to aerial feeding but rictal bristles are present in both aerial feeders (e.g. tyrant flycatchers and nightjars) and ground feeders (e.g. kiwis). However, their superficial resemblance with mammalian whiskers and the fact that the bristles are dead feather cells, enclosed at the base by an innervated follicle suggests that these are similar to mammalian whiskers (Lederer, 1972; Pass, 1989). Furthermore, rictal bristles may be capable of movement as several muscles are known to connect feather follicles together (e.g. smooth apterial muscle (counteracting horizontal movements of feathers), the smooth erector muscle (which lifts the feather up) and the depressor muscle (which pulls the feather down and counteracts the vertical rotation of feathers induced by airflows) (Ostmann *et al.*, 1963). Rictal bristles may therefore have a tactile function analogous to mammalian vibrissae (Lederer, 1972; Seneviratne and Jones, 2008).

Mammalian whiskers are known to transmit environmental information and play an important role in guiding navigation in dark, complex environments and aiding in prey capture (Dehnhardt et al., 1999; Anjum et al., 2006; Sarko et al., 2015). Rictal bristles may also function either as a funnel or a net, by increasing the size of the mouth gape and enhancing the efficiency of prey capture or to protect the eyes from vegetation and other particles while foraging (Keast and Saunders, 1991; Cunningham et al., 2011). However, to date, protection of the eyes is the only hypothesis truly confirmed by observational and experimental evidence using aerial insectivorous birds (Conover and Miller, 1980; Cunningham et al., 2011).

Despite the presence of mechanoreceptors within the rictal bristle follicles of some bird species (owls and kiwis), questions remain over their presence and functionality. Whilst the long tactile rictal bristles of kiwis are thought to help with nocturnal foraging and navigation (Corfield *et al.*, 2014), rictal bristles have yet to be associated with prey capture directly.

Several tyrant flycatcher species forage without using their rictal bristles as birds have been filmed catching their prey with their bill tips rather than at the base of the bill nearer to the mouth (Lederer, 1972). Therefore, perhaps birds with the same bill features as flycatchers and non-aerial feeders do not utilise rictal bristles while foraging. Although other aerial feeders, such as nightjars, swallows, and swifts, have larger gapes, it seems unlikely that rictal bristles play a role in prey capture (Lederer, 1972). Nonetheless, this has led some authors (e.g. Lederer, 1972) to suggest that rictal bristles could carry out a sensory function, but in order to investigate the likely sensory function of rictal bristles, firstly their follicle anatomy needs to be described to identify the presence of mechanoreceptors, Herbst corpuscles, as well as muscle fibres.

2.6.2 Preliminary study

In a previous study (MRes Thesis: Delaunay, 2016), I characterised bristle morphology in the nightjars and their relatives, and the bristle follicle anatomy in two species of nightjars, the fiery-necked nightjar (*Caprimulgus pectoralis*) and the pennant-winged nightjar (*Caprimulgus vexillarius*). The anatomy study revealed the presence of intrinsic fibres at the base and around the follicle of both species. Those fibres appeared to connect the rictal bristle follicles together (Figure 2-10, Figure 2-11). However, there were also differences between the two species. Muscle fibres were clearly identifiable (red) at the base and around *C. vexillarius* follicles (Figure 2-11) but not in *C. pectoralis*. The latter had thicker, blue, connective fibres at the base of its rictal bristles follicles (Figure 2-10). Their blue colour suggests that they might be collagen fibres. Moreover, *C. pectoralis* possessed tactile corpuscles, namely Herbst corpuscles at the base and around its bristle follicles, of which no evidence of presence can be observed in *C. vexillarius* (Figure 2-10; Figure 2-11). These findings suggest that rictal bristles may function as tactile sensors, at least in *C. pectoralis*. Variation of morphological characters observed within the Caprimulgiform order phylogeny (Figure 2-12) suggested that rictal bristles morphology may not simply be related to phylogeny but may have undergone changes due to common ecological pressures, which therefore could be better predictors to rictal bristle morphology across species. However, the link between bristle traits, ecology and phylogeny needs to be better explored in more species. This thesis will characterise rictal bristles in different ways, by describing their anatomy, morphology, developmental schedule, associated neuroanatomy, evolution and associated species life-history traits, in a range of different species (Caprimulgiformes and owls) to obtain a better understanding of their function.

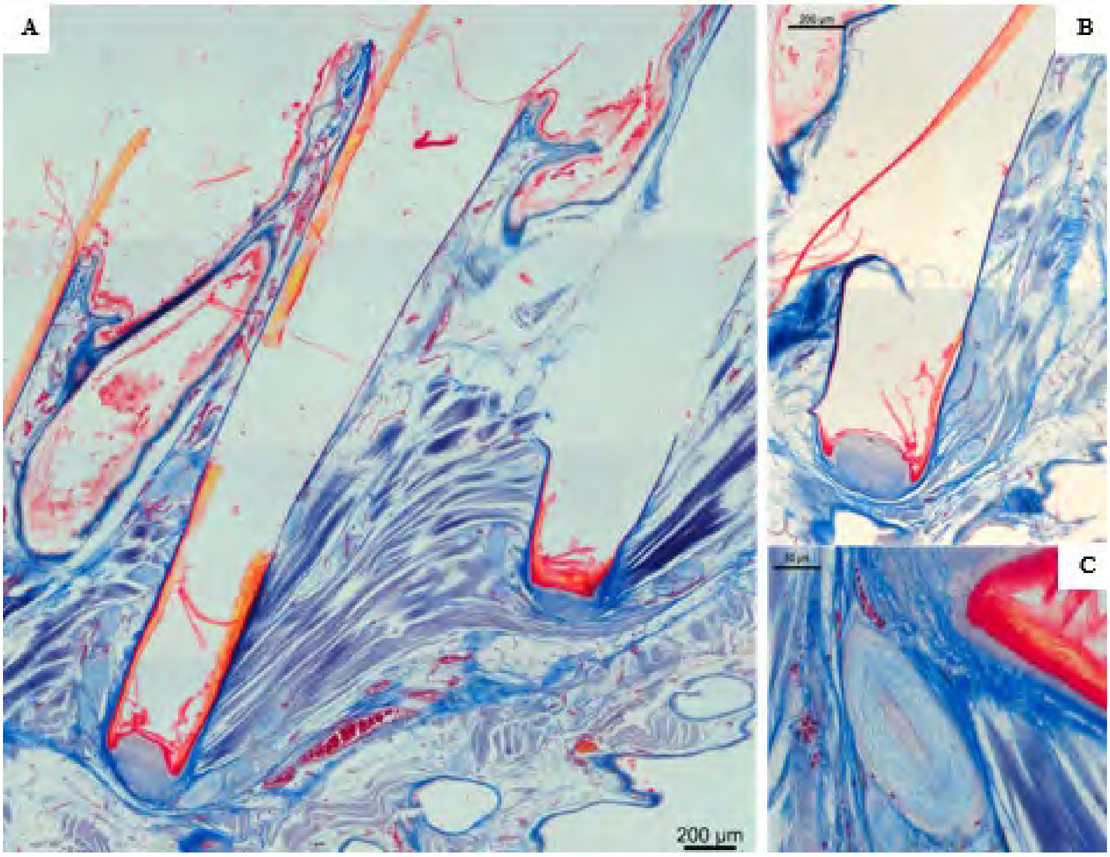


Figure 2-10. *Caprimulgus pectoralis* rictal region illustrating the bristle follicle anatomy, stained with Masson's trichrome. **A)** Row of rictal bristle follicles connected with intrinsic fibres (dark blue). **B)** Zoom showing the Herbst corpuscles in the immediate surroundings of the follicle. **C)** Enlargement on a Herbst corpuscle near the follicle.

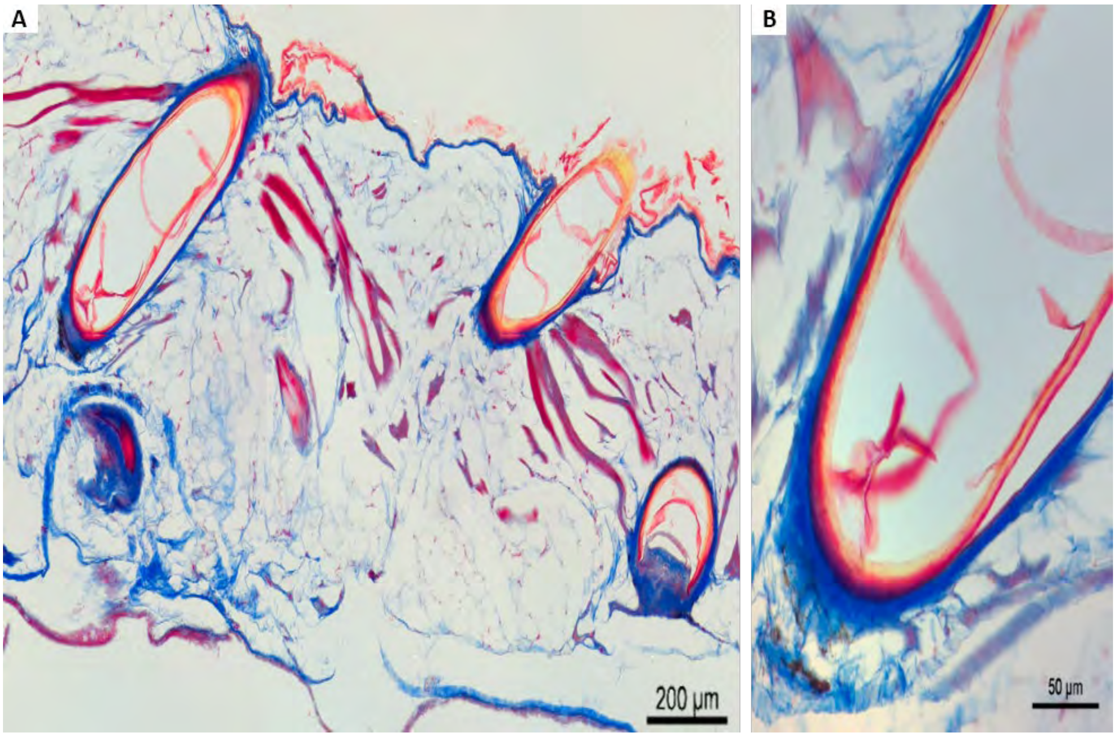


Figure 2-11. *Caprimulgus vexillarius* rictal region illustrating the bristle follicle anatomy, stained with Masson's trichrome. **A)** Row of bristles follicles connected with muscle fibres (red). **B)** Enlargement of a base of bristle follicle showing the absence of Herbst corpuscle in its surrounding.

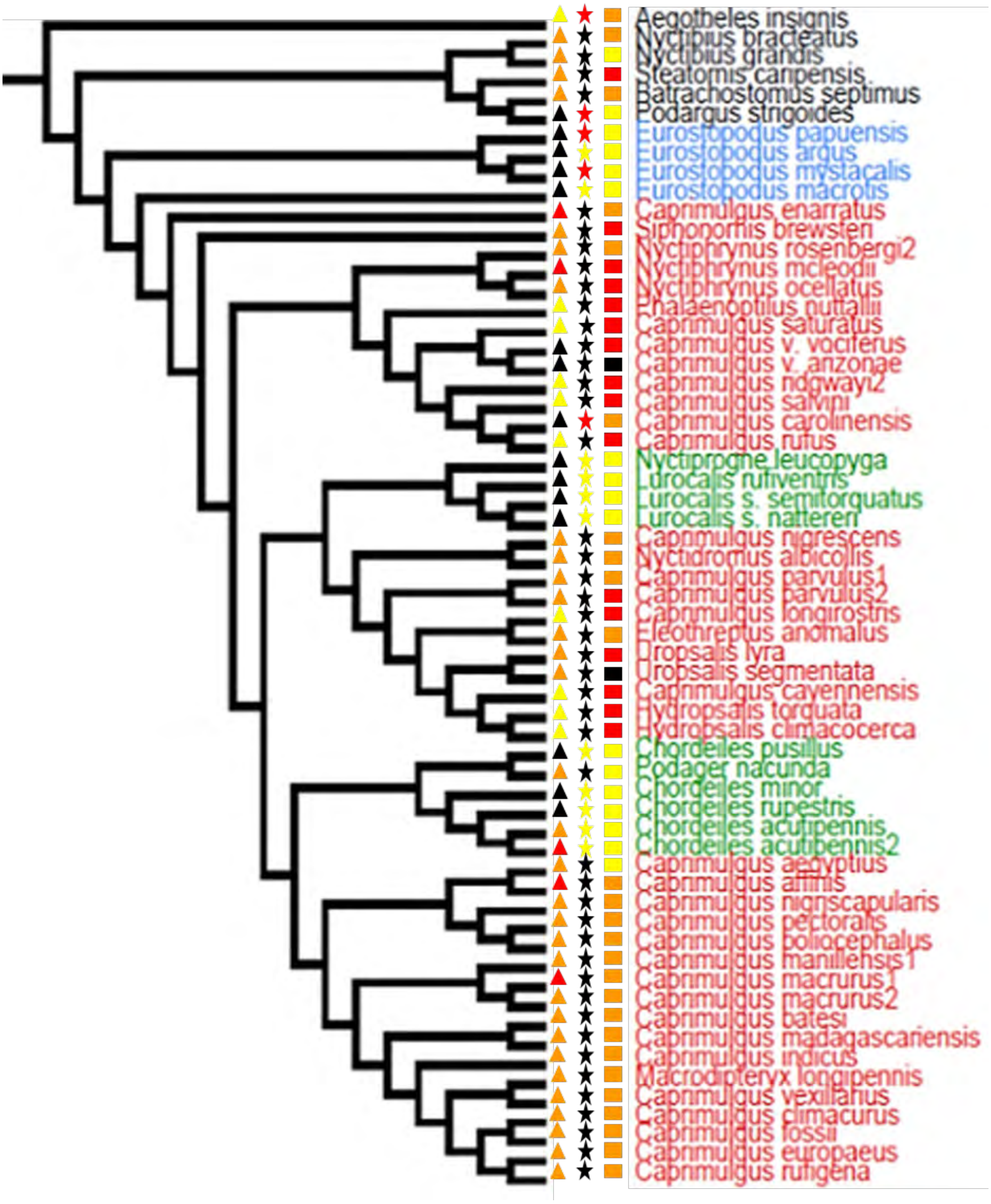


Figure 2-12. Consensus phylogenetic tree of the Caprimulgiformes order based on their genomic (C-CYTB, C-MYC and GH). Species in black represent the outgroup species, blue represents the Eurostopodinae, red represents the Caprimulginae and green represent the Chordeilinae. Triangles, stars and rectangles display rictal bristles morphological characteristics: stiffness, branching, length, respectively. The colour of the symbols illustrates the different states of these morphological characteristics. Likely ancestral state (0) is in yellow, state 1 is orange, state 2 in red and state 3 in black for stiffness and length (triangle, rectangle), while state 1 is in red and state 2 is in black for branching characteristics.

2.6.3 Key species considered in this thesis

A broad range of over 1000 avian species

As rictal bristles have been so far overlooked in the research, the number of species that have rictal bristles and their morphology are still unknown. Through recording the presence of rictal bristles and the description of bristle morphology of museum specimens of over 1,022 avian species, including 91 families and 29 orders many bird species, this PhD will build a large phylogenetic tree that illustrates the evolution of the rictal bristles in extant birds and will investigate the association of bristle presence and morphology with ecological traits, especially foraging traits.

Focussing on Caprimulgiformes

As introduced in chapter 1, mammalian whiskers are tactile and tend to be associated with nocturnal activity. The preliminary study (Delaunay, 2015) indicated that the period of activity, specifically nocturnality, might be associated with the presence of mechanoreceptors around rictal bristle follicles. Therefore, in this thesis, further follicle anatomy work will be conducted in species across the nocturnality spectrum (cathemeral/partially diurnal to obligate nocturnal). The species chosen for this work are all within Caprimulgiformes and their relatives, for several reasons: i) species within the order have distinctive facial bristles and noticeable rictal bristles along their beaks (Lederer, 1972), ii) this order is a relatively understudied, iii) the order is a diverse group containing a relatively high richness of species, found across many biomes globally (Sigursson and Cracraft, 2014), iv) this order includes species that forage across the nocturnality spectrum, v) these samples will continue to expand on the original, preliminary study. Furthermore, species such as oilbirds, potoos, frogmouths, nightjars and their relatives (Dyke and Van Tuinen, 2004) date back to the early Eocene, according to fossils found in North America and Europe (Dyke and Van Tuinen, 2004) and form part of the super order *Strisores* (Figure 2-1), which underwent a shift from diurnal to nocturnal ecology during their evolution (White, 2017). They are, therefore, an interesting group from an evolutionary point of view. While the Caprimulgiform order is an ideal group of species to study, spirit specimens are not abundant in museum and so, only a small subset of specific species (12) will have their rictal morphology and anatomy described in this thesis. Nevertheless, the anatomy work conducted in this thesis will be the first descriptions of rictal bristle morphology and anatomy, and neuroanatomy of Caprimulgiform species.

Barn owls (Tyto alba) and Tawny frogmouths (Podargus strigoides)

Little is known about the development of Caprimulgiform species, and nothing is known about the development schedule of the rictal bristles. However, other closely related nocturnal species such as owls, and particularly *T. alba*, have been more extensively described in the literature, including their chick development. *T. alba* is therefore an ideal comparison species to a lesser known Caprimulgiform species, *P. strigoides*, which was specifically chosen for two reasons: i) they are present in zoos, which make them accessible to study and ii) they are one of the biggest species in that order, which might make rictal bristles easier to observe in live birds.

2.6.4 Key concepts for the phylogenetic comparative methods used in this thesis

Characterising patterns of rictal bristle diversity, in terms of presence and morphology, at macro-evolutionary scales requires a phylogenetic perspective. Phylogenetically-related species tend to share phenotypical and ecological traits, therefore species can not be considered to be independent (Felsenstein, 1985; Harvey and Pagel, 1991). Accordingly, phylogenetic comparative studies that attempt to reconstruct the way a trait changes through time, must account for non-independence in the data, i.e. phylogenetic relationships between species (Felsenstein, 1985; Harvey and Pagel, 1991). To assess the accumulation of phenotypic variation across the phylogeny, transition rate models are used. Of all transition rate models, the Brownian motion model (BM) is typically used (Adams and Collyer, 2019), especially for continuous variables. Brownian motion is equivalent to an unbiased random walk, i.e. a change at each step is random with respect to other steps and has an equal chance of moving in a positive or negative direction, therefore BM is a drift model (Felsenstein, 1973, 1981). Other models of evolutionary transition rates exit: for continuous variables, there is the Ornstein-Uhlenbeck model (OU) - that incorporates both selection and drift (Hansen, 1997; Butler and King, 2004) - and the Early-Burst model (EB) - in which the rate of evolution increases or decreases exponentially through time (Harmon et al., 2010). For discrete variables, there are the one-parameter equal-rates model (ER), the symmetric model (SYM) - in which the forwards and reverse transitions between states are constrained to be equal -, and the all rates different model (ARD) - in which all possible transitions between states receive distinct parameters (Paradis, 2012).

Three fundamental evolutionary assumptions of the phylogenetic comparative methods should be evaluated in all phylogenetic analyses: i) phylogenetic uncertainty, ii) phylogenetic signal (i.e. closely related species tend to present more similar characters than expected by chance), and iii) validating the model by comparing the best fitted evolutionary model and evaluating the uncertainty of the estimated model parameters (Hernández et al., 2013). Phylogenetic uncertainty is based on the fact that phylogenies are usually built from groups of morphological and molecular data (Felsenstein, 2004), which are themselves subject to error and uncertainty (Revell et al., 2005). High uncertainty in phylogenetic relationships between species can lead to different phylogenies giving different results to the same comparative questions, therefore all conclusions derived from the single used of a phylogeny in a comparative analysis are conditional upon that particular selected phylogeny (Hernández et al., 2013). Phylogenetic signal is assessed using Pagel's λ, which is a scaling factor implemented so that the tree fits Brownian motion, and is usually ranging from 0 to 1, where 0 means no phylogenetic structure. In other words, this parameter denotes the phylogenetic signal of each trait, with the trait distribution being independent of the phylogeny when $\lambda=0$ and fits the phylogeny as expected under Brownian motion when $\lambda= 1$ (Pagel, 1999). Finally, the best-fitted model is assessed by comparing the models together using the Akaike Information Criterion (AIC) (Butler and King, 2004), which estimates the quality of each model in comparison to the other models (Akaike, 1973, 1974, 1978; Burnham and Anderson, 2002).

In this thesis, I will be using the ancestral state character reconstruction (ASR) to retrace rictal bristle presence and morphology evolution. ASR is indeed an essential tool for exploring the evolutionary relationships amongst individuals, population or species to their ancestors (Royer-Carenzi et al., 2013; Joy et al., 2016). It is a useful tool to apply to discover how traits evolved and to understand function (Pagel, 1999). The ASR method combines information about evolutionary relationships from a given phylogenetic tree with character states at each branch tip (e.g. an extant species) (Joy et al., 2016). The second method that will be used in this thesis to investigate the association between the rictal bristle presence, morphology and species-specific ecological traits (e.g. foraging traits, period of activity), is a phylogenetic generalised linear mixed model that employs a Bayesian approach (MCMCglmm) (Hadfield and Nakagawa, 2010; Hadfield, 2015). Bayesian method using Markov Chain Monte Carlo (MCMC) statically samples phylogenies from the probability distribution (Larget and Simon, 1999; Huelsenbeck et al., 2000, 2001; Holder and Lewis, 2003). This method is flexible (implementation of random and fixed effects), accounts for non-independence in the data (Hadfield, 2010), and also accounts for phylogenetic uncertainty by estimating the parameters of interest in each tree and integrating the estimations over all the trees given a sample of probability distribution of phylogenetic trees (Pagel et al., 2004).

2.7 Thesis rationale

2.7.1 Gaps of knowledge

The gaps in knowledge identified from the literature includes:

i) There is not a comprehensive record of the presence/absence of rictal bristles and their form in many species

ii) Rictal bristle follicle anatomy has only been described in kiwis and some species of owls

iii) Rictal bristle presence and development in chicks are unknown, as well as if these vary between species.

iv) Occurrences of appearance/disappearance of rictal bristles in species, families, and order throughout avian evolution is unknown

v) Ancestral character state of rictal bristle presence and form is unknown

vi) Potential associations of rictal bristle presence and form with ecological traits, notably foraging related traits, such as period of activity, foraging method and height, habitat density and diet, are unknown

vii) Potential associations of rictal bristles presence and morphology with brain areas, notably mechanosensory associated, are unknown

2.7.2 Specific hypothesis and predictions

The specific hypotheses and predictions generated by my literature review, that this thesis will address, includes:

- **Rictal bristle morphology will vary across species**

Species with long, thick rictal bristles are expected to present more numerous mechanoreceptors around their rictal bristle follicles, compared to species with smaller bristles or semi-bristles.

- **Variation in rictal bristle morphology will be associated with specific ecological traits**

Rictal bristle shape and anatomy are expected to be associated with ecological traits, such as nocturnality; such that nocturnal species may have longer, thicker bristles and a higher number of mechanoreceptors within their follicles.

- **Rictal bristles will be present in hatchlings and play a role in the developing young**

Chicks have mechanoreceptors that are fully developed from birth but they undergo different plumage throughout their development, and the development of the feather is species-specific. Mammalian whiskers are essential in the early development of the pups, playing a role in navigation, huddling and feeding. Therefore, rictal bristles may also play an important role in the development of the chick. Rictal bristles are expected to develop relatively early in chick morphogenesis and be involved in feeding behaviour.

- **Rictal bristles will be present in a common avian ancestor**

As early-feathers are thought to have been simple bristles, it would be expected that the plesiomorphy of rictal feathers in the most recent common ancestor (MRCA) of birds might have been bristles.

- **Rictal bristle presence and form will vary within species, families and order**

Avian phylogeny has undergone many morphological modifications throughout evolution, therefore several events of rictal bristle disappearance and gain would be expected.

- **Rictal bristle form and function will be associated with foraging**

Feathers evolved in different forms, shapes, sizes and textures throughout evolution. Therefore, the expected variation in rictal bristle morphology amongst species may be associated with ecological traits and so vary in function according to the bird's ecology. More prominent rictal bristles would be expected to have a tactile function, especially in species that forage in dark, complex environments.

- **Rictal bristle presence and morphology will be associated with mechanosensory associated brain areas**

Species having mechanoreceptors in the surroundings of their rictal bristle follicles might have a larger rostral fraction of the Wulst as well as other somatosensory areas, such as the thalamic nuclei (i.e. Bas and PrV).

2.7.3 Aims and objectives

In this thesis, I will characterise rictal bristle form and function following Tinbergen's framework by describing:

i) How rictal bristles work, including bristle morphology and follicle anatomy
ii) How rictal bristles develop
iii) How rictal bristles evolved
iv) The functions of rictal bristles
v) How rictal bristles work, including the associated mechanosensory brain areas

I will investigate rictal bristles in a number of avian species and focus on nightjar, owl, and frogmouth lineages, which were selected due to their diversity, nocturnality and the prominence of their facial bristles.

Chapter 3

Anatomy of avian rictal bristles in Caprimulgiformes

This chapter is based on the publication: **Delaunay, M.G**., Larsen, C., Lloyd, H., Sullivan, M. and Grant, R.A. (2020). Anatomy of avian rictal bristles in Caprimulgiformes reveals reduced tactile function in open-habitat, partially diurnal[1] foraging species. Journal of Anatomy.

[1] In this thesis, "partially diurnal" species will be referred to as nocturnal cathemeral (predominantly nocturnal species that occasionally feed during daytime from late morning to late afternoon). A more detailed description of activity periods will be added in Chapter 7, i.e. diurnal species and diurnal cathemeral species.

Chapter summary:

Avian rictal bristles are present in many species of birds, especially in nocturnal species. However, it is not yet known if avian rictal bristles have a sensory function, and their morphology, anatomy and function have also not been described in many species. This study compares bristle morphology, follicle anatomy and their association with foraging traits, across twelve Caprimulgiform species. Rictal bristle morphology and follicle anatomy were diverse across the twelve species. Nine of the twelve species had mechanoreceptors around their bristle follicles; however, there was a large variation in their musculature, mechanoreceptor numbers and bristle morphology. Overall, species with short, thin, branching bristles that lacked mechanoreceptors tended to forage pre-dusk in open habitats. Whereas species with mechanoreceptors around their bristle follicle tended to forage at night and in more closed habitats. I suggest that rictal bristles are likely to be tactile in many species and may aid in navigation, foraging and collision avoidance; however, identifying rictal bristle function is challenging and demands further investigation in more species.

3.1 Introduction

The prevalence of bristles in nocturnal and crepuscular bird species suggests that rictal bristles may be tactile, and play a role in navigation and obstacle avoidance in low-light conditions (Lucas and Stettenheim, 1972), but evidence for this is scant. Anatomical studies conducted on the rictal bristles of owls (*Bubo bubo, Asio flammeus, Athene noctua* and *Strix aluco*) have revealed that their follicles are connected by muscle and connective tissue within the dermis, and associated with nerves, and mechanoreceptors (Küster, 1905; Stettenheim, 1973). Similarly, the follicles of the rictal bristles of Brown kiwis are surrounded by Herbst corpuscles (Cunningham et al., 2013), which are vibration-sensitive mechanoreceptors. The presence of Herbst corpuscles indicates that rictal bristles are likely to be sensitive to touch, airflow and vibrations. Feather follicles are connected to each other by several muscles, notably by the smooth apterial muscle (counteracting horizontal movements of feathers), the smooth erector muscle (which lifts the feather up) and the depressor muscle (which pulls the feather down and counteracts the vertical rotation of feathers induced by airflows) (Ostmann *et al.*, 1963). Bristle follicles may also be connected to each other and have the capacity to be mobile; however, bristle anatomy has been described in only a handful of bird species, and we know very little about rictal bristle mechanoreceptors and musculature. Further histological work is therefore required to describe rictal bristle anatomy and to test hypotheses about their function.

In mammals, longer and more numerous whiskers are found in small, nocturnal, arboreal species. These species have regularly arranged whisker follicles with large, regular intrinsic muscles (Muchlinski et al., 2013, 2020; Grant et al., 2017); longer, thicker and stiffer whiskers also tend to have more numerous mechanoreceptors (Ebara *et al.*, 2002). While the anatomy of mammalian whiskers is well described, the morphology and anatomy of avian rictal bristles are relatively unknown, and the relationship between bristle morphology and anatomy has not previously been investigated.

This study will test the hypotheses described in Chapter 2:

- **Rictal bristle morphology will vary across species**

Species with long, thick rictal bristles are expected to present more numerous mechanoreceptors around their rictal bristle follicles, compared to species with smaller bristles or semi-bristles.

- **Variation in rictal bristle morphology will be associated with specific ecological traits**

Rictal bristle shape and anatomy are expected to be associated with ecological traits, such as nocturnality; such that nocturnal species may have longer, thicker bristles and a higher number of mechanoreceptors within their follicles.

I focus on bird species belonging to the Order Caprimulgiformes, commonly referred to as nightjars, which are widely known for their nocturnal ecology, with species exhibiting a highly diverse range of foraging traits. Many Caprimulgiform species have obvious and prominent rictal bristles that vary in their shape, length, number and thickness. Here I describe the bristle morphology and follicle anatomy of 12 species, representing all five families and nine of the 22 genera of the traditional Caprimulgiformes (Cleere, 1998). I go on to describe the follicle anatomy, by identifying the presence and prevalence of mechanoreceptors Herbst corpuscles and provide muscle fibre descriptions. I explore associations with bristle morphology traits (bristle length, width and number) and discuss our findings in light of recent data on phylogenetic relationships and known foraging traits (i.e. period of activity, foraging method and height, diet and habitat density) that I have hypothesised associated with rictal bristle form and function.

3.2 Material and methods

3.2.1 Samples

Rictal bristle morphology and follicle anatomy were described in twelve species belonging to the Caprimulgiformes order in this study: fiery-necked nightjar (*Caprimulgus pectoralis*), pennant-winged nightjar (*Caprimulgus vexillarius*), European nightjar (*Caprimulgus europaeus*), spotted nightjar (*Eurostopodus argus*), Australian owlet-nightjar (*Aegotheles cristatus*), nacunda nighthawk (*Chordeiles nacunda*), common nighthawk (*Chordeiles minor*), tawny frogmouth (*Podargus strigoides*), large frogmouth (*Batrachostomus auritus*), Gould’s frogmouth (*Batrachostomus stellatus*), oilbird (*Steatornis caripensis*), pauraque (*Nyctidromus albicollis*). Only the aforementioned species within the Caprimulgiform order were dissected, as they were the only ones that we had access to within the collection. These particular species were also in high numbers within the collection (>3 individuals) so I had the authorisation to dissect them. *C. Pectoralis* and *C. vexillarius* specimens were donated by Professor Tim Birkhead at the University of Sheffield; they were decapitated and preserved in formalin prior to their donation. All the other specimens were from the spirit collection at the Natural History Museum, Tring, UK, where they were dissected and preserved in 4% Paraformaldehyde (PFA) in Phosphate Buffer Solution (PBS). The rictal bristle region was fully intact in all specimens, therefore it was representative of the morphological characteristics of each species, with the exception of *B. auritus*, whose bristles were damaged prior to collection. All work in this study was approved by the local ethics committee at Manchester Metropolitan University.

3.2.2 Bristle morphology

For each species, the three longest rictal bristles were scanned on an Epson V600 scanner (12800 dpi). The three longest bristles were chosen for measuring as they represent how the maximum area the bristles may occupy, and there was no way to know if shorter bristles were still growing or had been damaged. Photos were taken using the Epson scan v 3.9.2 software, which gave calibrated measurements of the bristles. We were unable to photograph the bristles of *B. auritus* due to some of the bristles being damaged, and consequently, we scanned only the base of rictal bristles for this species (see Figure 3-3). Bristle total length, and bristle width at the base and tip were measured using the segmented line tool on ImageJ software from the scanned images. To compare the different morphotypes of bristles across species, I used three categories of bristle length and bristle width (Table 3 -1).

Table 3-1. Different categories of bristle morphology (based on the measurements of the bristles on ImageJ) and bristle number.

Bristle features	Categories	Measurements
Bristle length (mm)	Short	<10
	Medium	15 to 30
	Long	>30
Bristle width	Thin	< 0.3
	Relatively thick	0.3 to 0.6
	Thick	> 0.6
Bristle number	Few	≤10
	Many	>10

Bristle number was counted from the rictal region of specimens from the skin collection within the Natural History Museum, Tring and Liverpool World Museum. Only the best-preserved specimens were chosen for this study to ensure accurate measurements of bristle number, which would not have been altered by the process of skin removal and specimen conservation. Bristle number very rarely varied between each side of the head in each specimen. In the rare occurrences where it did vary, which was only by 1-2 bristles, I reported the maximum bristle counts for each species. Bristle number was defined in two categories: few - when 10 bristles or lower bristle were present, and many - when above

10 bristle were present (Table 3-1). Two specimens per species were measured for 8 of the 12 species examined. Due to labelling (former species names or labelling error), *P. strigoides* and *N. albicollis* had four specimens measured, and *E. argus* and *C. minor* had three. Attempts were also made to measure adult specimens of each sex; however, this was not always possible, since some specimens were either not labelled, or incorrectly labelled, resulting in 4 of the species where each sex was measured (*P. strigoides, C. nacunda, C. europaeus,* and *N. albicollis*), 1 species where both specimens were male (*C. pectoralis*), 2 species where one specimen was labelled (male) and the other(s) unknown (*C. vexillarius* and *E. argus*, respectively), 1 species for where one of the specimens were female, and one unknown (*C. minor*). A total of four species were measured from unknown sex individuals (*B. auritus, B. stellatus, A. cristatus, S. caripensis*. No significant differences were observed in the four species for which I had a male and a female measured in this study: presence (Pearson's χ^2, x-squared=0, df=1, p=1), shape (Pearson's χ^2, x-squared=0, df=1, p=1) and number (Wilcoxon test, W=9.5, p = 0.766) of rictal bristles; therefore, rictal bristles are likely to be sexually monomorphic.

3.2.3 Follicle anatomy

Dissection and histology

I define the rictal region as the patch of skin from the rictus to nares, at the base of the upper mandible of the beak (Figure 3-1). Subsequently, I defined all bristles collected from this area as rictal bristles. All other (non-bristle) feathers were trimmed from the rictal region of each specimen. The rictal bristle region was removed from one side of the head by cutting a 5mm wide piece of skin along the length of the upper beak and across the head near the nare using a scalpel (Figure 3-1). There was a noticeable variation in bristle positioning across all specimens examined, and consequently, the shape and position of the dissected sections varied. Rictal bristles were removed from the skin of each sample by cutting them flat to the skin sample; the base of the bristle was kept within the follicle to allow the follicle to maintain its form during slicing and staining.

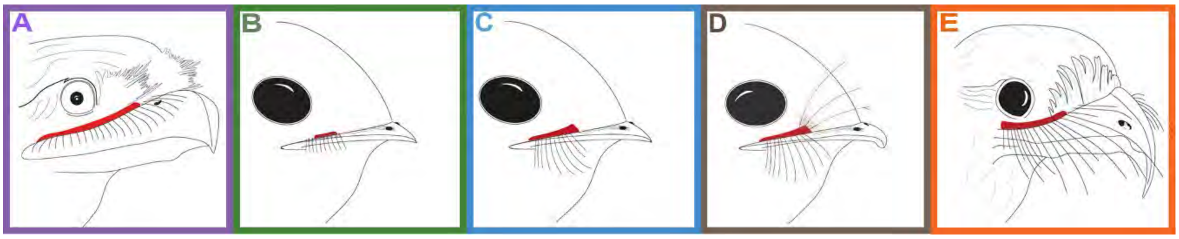


Figure 3-1. Rictal region with patch of skin that has been removed for the experiment (red)**.** Different groups shows the different bristle morphotypes in our Caprimulgiform species, and their beak shapes. Following their rictal bristles morphology, the different groups contain A) *Podargus strigoides, Batrachostomus auritus and B. stellatus*; B) *Eurostopodus argus, Chordeiles nacunda and C. minor*; Group C) *Caprimulgus pectoralis, C. europaeus, C. vexillarius, Nyctidromus albicollis*; Group D) *Aegotheles cristatus* and Group E) *Steatornis caripensis*. The red patch illustrates the rictal region and so the patch of skin that has been removed for the experiment.

Skin tissue was flattened for five hours between two sponges in histology cassettes in 70% industrial methylated spirit (IMS). Tissue samples were loaded into a tissue processor (Shandon Citadel 2000) to dehydrate through a graded series of ethanol (70, 80, 90 and 100%) and xylene baths and infiltrated with paraffin wax, in a process lasting approximately 20 hours (Figure 3-2). The tissue samples were then embedded in solid blocks of paraffin that were sliced at 10µm on an automated rotary microtome (Thermo-scientific microm HM355S) with a water bath (35-37°C), in which the floating slices were collected by mounting them on to slides and left to dry at 38°C overnight. These slides were then prepared for staining. They were put in a fixative solution (4% paraformaldehyde in 0.1 M phosphate buffer) for an hour and introduced to Bouin's solution for 4 hours. The slides were then cleared with xylene, rehydrated with ethyl alcohol (100, 90, 80, and 70%) and finally moved through the sequence of solutions for the Masson's Trichrome staining, i.e. Biebrich Scarlet acid, Phosphotungstic and Phosphomolybdic acids, Aniline blue, and acidified water baths, with multiple washes of distilled water between each stage. The slides were then dehydrated with ethyl alcohol (70, 80, 90, 100%), and xylene, towel dried (without touching the tissue) and cover-slipped with DPX mountant. All slides were visualised using a Zeiss AxioImager M1 Brightfield microscope, and microscope images were captured using the Zeiss Zen Pro imaging software and an AxioCam HMRc.

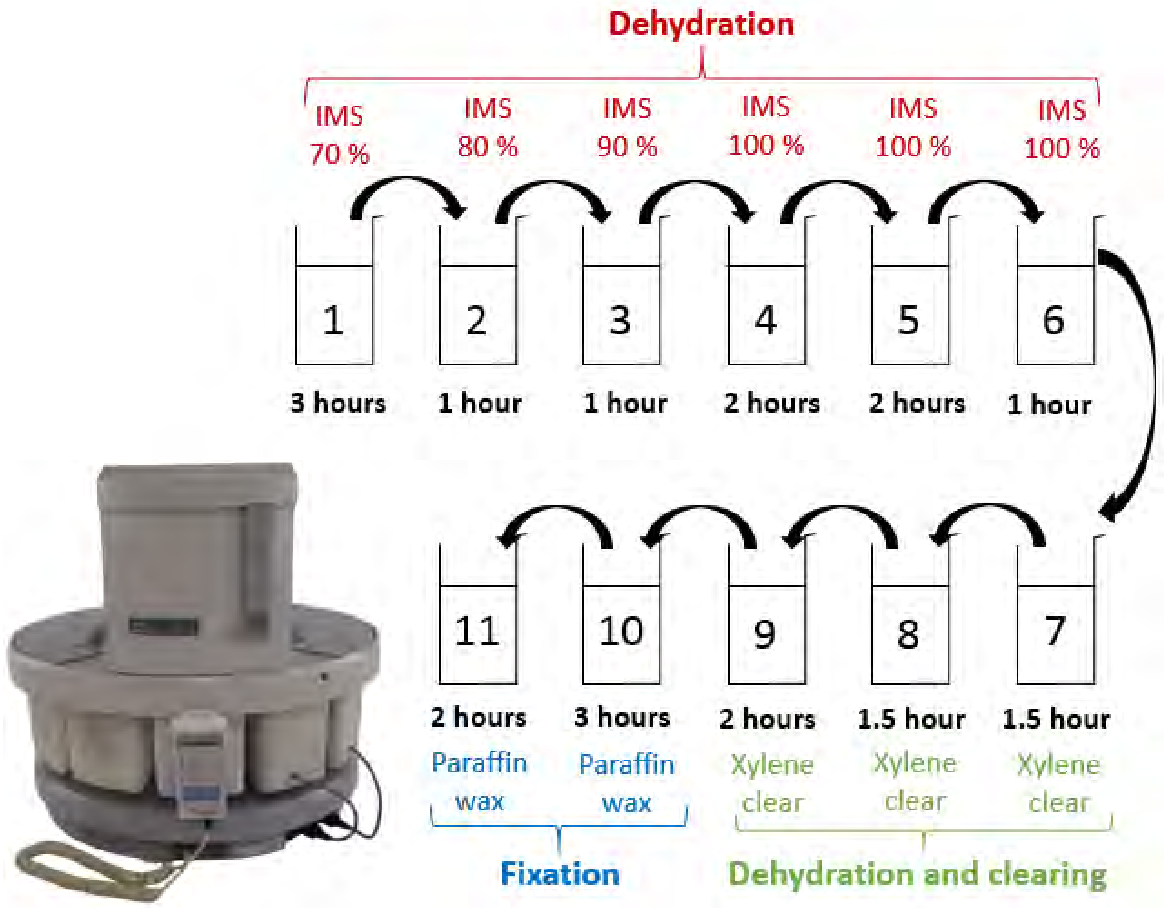


Figure 3-2. Tissue processor bath program of 11 steps (20 hours long, on the Shandon citadel 2000 tissue processor). The tissue processor is automated, moving tissue from graded bath of ethanol (Industrial methyl solution, IMS) to Xylene clear baths, and finally to paraffin wax baths. Ethanol displaces the water of the tissue while Xylene completes the dehydration and clear the tissue from ethanol. The paraffin wax infiltrates the tissue to preserve it (tissue fixation).

Characterisation of the follicle anatomy

For each species, components of the follicle and immediate surroundings of the follicle were identified and characterised from the microscope images, including describing the tissue type, musculature and mechanoreceptors. For each species, the dermis density and the adipose tissue quantity was quantified as possible predictors of tissue quality and functionality, using the following coding system (Table 3-2). The quantity of adipose tissue was expressed as the percentage of space allocated for adipose tissue within the integument section imaged. Dense tissue may correspond to a higher quality of tissue, containing more sensory components. For each species, the presence and type of intrinsic fibres and mechanoreceptors were identified from the dermis.

Table 3-2. Different categories of the anatomy feature reported throughout the histology images.

Anatomical features	Characteristics	Criteria	Measurements
Dermis density	**Dense**	Adipose tissue quantity (%)	≤ 40
	Porous		> 40
Fibre bundle size	**NA**	Number of adjacent fibres	0
	Small		> 5
	Large		≥ 5
Herbst corpuscle quantity	**Low**	Number of HC at the follicle	< 7
	High		≥ 7

Types of intrinsic fibres were determined following the feather muscle description by Homberger and De Silva (2000) and defined as smooth apterial muscle, and erector or depressor feather muscle (the histology sections showed the characteristic diagonal fibres of the erector and depressor muscles, running from the tip of a feather follicle to the base of the neighbouring one, but the angle of the sections did not permit us to identify which one of the depressor or erector was present on the picture). The size of the fibre bundles was categorised by counting the number of fibres attached together in the area immediately surrounding the follicle (Table 3-2). The presence and absence of mechanoreceptors was documented, and where present, the number of mechanoreceptors were counted and defined per follicle (Table 3-2). Mechanoreceptor position was described as "*base*" when they were only apparent at the proximal tip (base) of the bristle follicle, or "*around*" when they were recorded from the base and the sides of the follicle. Mechanoreceptors were identified as Herbst corpuscle following Gottschaldt (1985). It was not possible to measure the depth of the bristle follicle due to variation in the orientation of the follicles within the tissue sample and the resulting histology slices.

Relationship between bristle morphology, anatomy, and foraging traits

Phylogenetic relationships of all nightjar species based on rictal bristle morphotypes (cf. drawings on phylogenetic branches in Figure 3-4) were represented using a phylogenetic tree from Birdtree.org (http://birdtree.org, tree set: HackettStage2Full, sample size: 1000). Two k-means cluster analyses were conducted in Matlab (MATLAB and Statistics Toolbox Release 2019a, The MathWorks, Inc., Natick, Massachusetts, United States) to identify morphotypes from our bristle morphology and follicle anatomy descriptors. The bristle morphology variables included the discrete categories of bristle length, width, number and branching, and the follicle anatomy variables included the discrete categories of muscle bundle size, tissue density and Herbst corpuscle number. Both datasets were partitioned into five defined groups using k-means distance measures. Relationships between the numbers of Herbst corpuscles, and bristle length, width and number were visualised using the package *ggplot2* (Wickham, 2009) in R software, version 3.5.0 (R Core team, 2016) and analysed using non-parametric Spearman's rank correlations (due to low sample sizes and skewed data). All values are presented mean values ± SD with significance level assumed at $P<0.05$. Five ecological traits from Cleere (1998) were identified for each sampled species (Table 3-3) in order to compare and discuss these ecological traits in association with bristle morphology and follicle anatomy. These traits included period of activity, diet, foraging method, foraging height and habitat density; they are defined in detail in the supplementary material (Table 3-3).

Table 3-3. Ecological factors of interest for each species of the study, based on Cleere (1998). Period of activity includes nocturnal cathemeral (bird active through the night as well as before dusk), crepuscular (birds active at dusk and dawn), and nocturnal (during the night). Diet comprises carnivore (birds eating insects, frogs, small mammals), insectivore (birds eating strictly insects), and frugivore (birds eating fruits). Foraging methods include foraging, which is plucking fruits (S. caripensis) or picking up food from branches or on the ground (B. auritus, B. stellatus), hawking (hunting on the wing), and sallying (leaping out from perches or the ground). Foraging heights cover low altitude (flying under the canopy), various altitude and high altitude (flying above the canopy). Habitat density consists of closed habitat (e.g. rainforest, woodlands), open habitat (e.g. open country, grassland, deserts, savannah), and semi-open (a mixture of open country and woodlands). Species names are colour-coded following their bristle morphotypes: frogmouths in purple, nighthawks and spotted nightjar in green, nightjars and pauraque in blue, Australian owlet-nightjar in brown, and oilbird in orange (Figure 3-1).

Species	Period of activity	Diet	Foraging method	Foraging heights	Habitat density
P. strigoides	Crepuscular and nocturnal	Carnivore	Foraging	Low	Closed habitat
B. auritus	Nocturnal	Insectivore	Foraging	Low	Closed habitat
B. stellatus	Nocturnal	Insectivore	Foraging	Low	Closed habitat
C. nacunda	Nocturnal cathemeral	Insectivore	Hawking	High	Open habitat
C. minor	Nocturnal cathemeral	Insectivore	Hawking	Various	Open habitat
E. argus	Crepuscular and nocturnal	Insectivore	Hawking and sallying	Low	Semi-open habitat
C. pectoralis	Crepuscular and nocturnal	Insectivore	Sallying	Various	Semi-open habitat
C. vexillarius	Nocturnal cathemeral	Insectivore	Hawking	High	Semi-open habitat
C. europaeus	Crepuscular and nocturnal	Insectivore	Hawking	Various	Semi-open habitat
N. albicollis	Crepuscular and nocturnal	Insectivore	Sallying	Low	Closed habitat
A. cristatus	Crepuscular and nocturnal	Insectivore	Hawking and sallying	Various	Closed habitat
S. caripensis	Crepuscular and nocturnal	Frugivore	Foraging	Various	Closed habitat

3.3 Results

3.3.1 Bristle Morphology

All three frogmouth species (*P. strigoides, B. auritus* and *B. stellatus*) had rictal bristles of medium length with a thick bristle base, and a high number of bristles (Figure 3-4), which were conspicuously branched in structure (Figure 3-3). *P. strigoides* and *B. auritus* bristles were branched throughout with barbs and barbules, especially at the base, while *B. stellatus* had simple branching bristles with numerous barbs throughout (Figure 3-3). *C. pectoralis, C. vexillarius, C. europaeus* and *N. albicollis* had medium length bristles with unbranched rictal bristles that occurred in lower numbers (10 bristles or less per side of the face) than the other species in the study (Figure 3-3, 4). In contrast, *C. nacunda, C. minor* and *E. argus* had the thinnest and shortest rictal bristles that occurred in high numbers, and were branched with barbs (Figure 3-3, 4). In all species with branched rictal bristles, *C. nacunda* had the least number of barbs on the shaft, from base to tip (Figure 3-3). *A. cristatus* had similar bristle lengths and widths to *C. pectoralis, C. vexillarius, C. europaeus* and *N. albicollis* (all with medium length and width), however, its bristles were a combination of branched with barbs from the base to the tip of the bristles and unbranched bristles, and had relatively lower bristle numbers (Figure 3-3, 4). Finally, *S. caripensis* had thick, long, unbranched rictal bristles that curved downwards, in comparison to the bristles on all the other species, which curved slightly upwards at the distal tip (Figure 3-3, 4) and had a high number of rictal bristles (15 bristles ± 1.4 SD) (Figure 3-4).

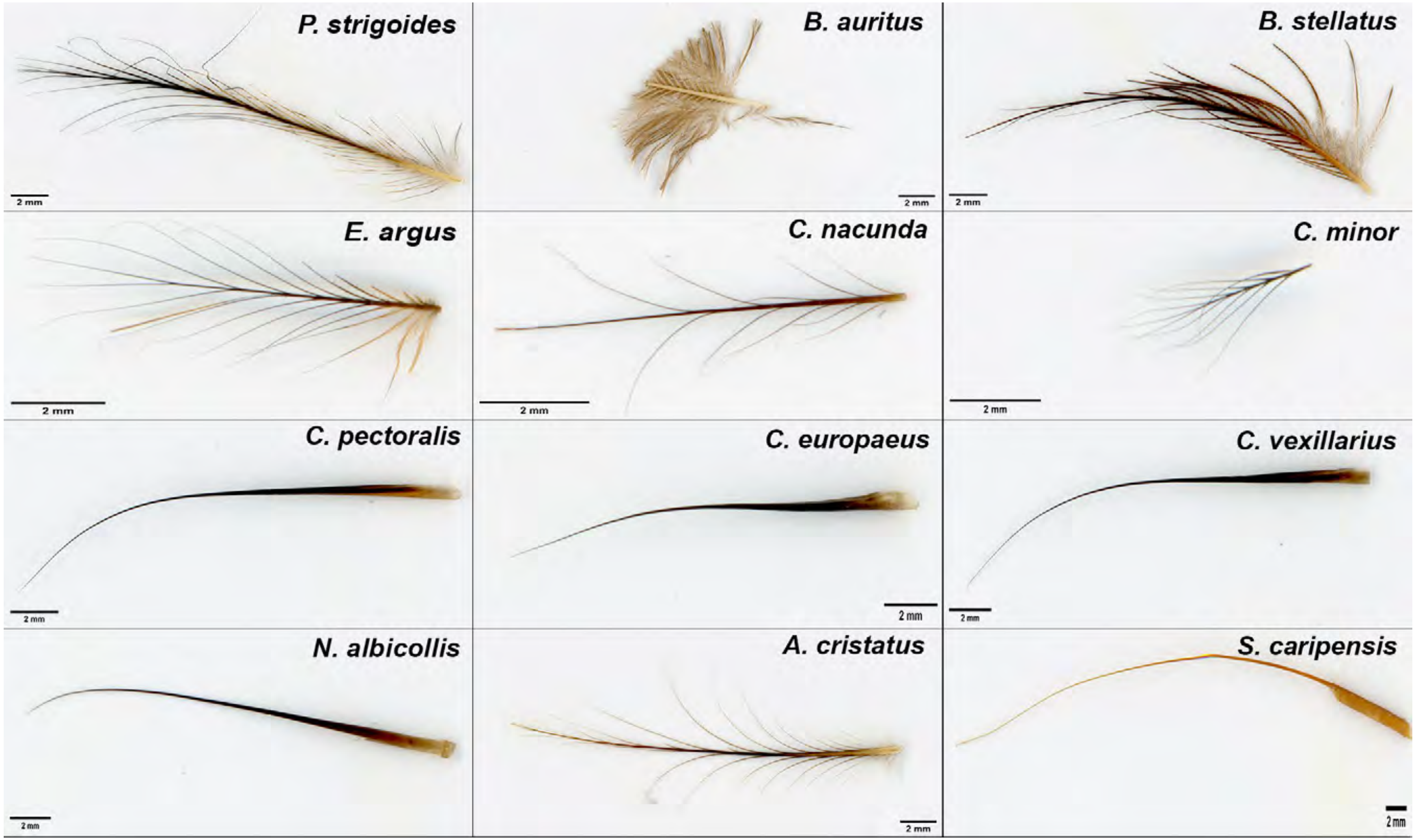
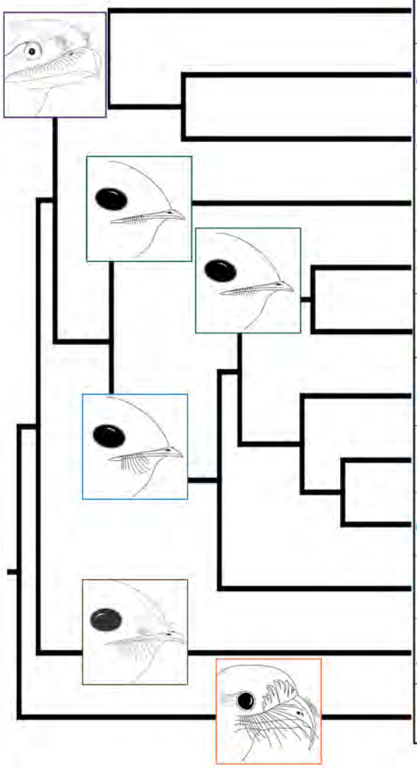


Figure 3-3. Scan of 12 rictal bristles showing the different bristle morphologies present in the Caprimulgiform order. Rictal bristles illustrated are from the longest rictal bristles found for each of our 12 species. Scale bar is 2 mm.



Species	Bristle length (mm)	Bristle width base (mm)	Bristle width tip (mm)	Bristle number
Pogardus strigoides	29.15 ± 2.24	0.45 ± 0.06	0.02 ± 0.01	20.8 ± 1.5
Batrachostomus auritus	NA	0.33 ± 0.30	NA	14 ± 1.4
Batrachostomus stellatus	21.28 ± 1.36	0.31 ± 0.02	0.04 ± 0.02	14 ± 0
Eurostopodus argus	9.58 ± 1.23	0.14 ± 0.02	0.02 ± 0.00	16 ± 2
Chordeiles nacunda	6.79 ± 0.61	0.14 ± 0.02	0.04 ± 0.01	13.5 ± 2.1
Chordeiles minor	5.10 ± 0.27	0.06 ± 0.01	0.07 ± 0.01	18 ± 0
Caprimulgus pectoralis	20.55 ± 0.59	0.58 ± 0.06	0.03 ± 0.00	10 ± 0
Caprimulgus europaeus	15.42 ± 0.18	0.48 ± 0.05	0.02 ± 0.00	9 ± 0
Caprimulgus vexillarius	15.83 ± 3.67	0.53 ± 0.06	0.03 ± 0.02	6.5 ± 0.7
Nyctidromus albicollis	21.17 ± 0.53	0.44 ± 0.08	0.03 ± 0.01	8.8 ± 1.3
Aegotheles cristatus	22.51 ± 1.10	0.45 ± 0.04	0.03 ± 0.01	11 ± 0
Steatornis caripensis	36.36 ± 7.84	0.41 ± 0.27	0.03 ± 0.02	15 ± 1.4

Figure 3-4. Phylogenetic tree for the species belonging to the Caprimulgiformes order used in this study, combined with a table showing their bristle measurements. Drawings showing the bristle morphology and position on the beak were added on the tree for better visualisation, and also to illustrate the head and beak shapes of the species. The tree was created by BirdTree.org. Measurements were taken from scanned bristles from each species and included the mean bristle length, mean bristle width at the base and at the tip (± standard deviation). Species names are colour-coded following their bristle morphotypes: frogmouths in purple, nighthawks and spotted nightjar in green, nightjars and pauraque in blue, Australian owlet-nightjar in brown, and oilbird in orange.

Therefore in our twelve species, bristle morphology could be visually summarised by five different morphotypes (coloured purple, green, blue, brown and orange in Figure 3-4), and was confirmed by a k-means cluster analysis (Table 3-4). Combining data from all the species sampled, bristle length and width were not correlated (N=11, Spearman's Rank: *rho*=0.36, *p*= 0.27; Figure 3-5), nor was bristle length correlated with bristle number (N=11, Spearman's Rank: *rho*=0.05, *p* = 0.88). However, bristle number and bristle width were correlated (N=12, Spearman's Rank: *rho*= -0.67, *p*= 0.016; Figure 3-5) suggesting that for the species with more bristles present, such as *C. nacunda, C. minor* and *E. argus*, also had much thinner bristles.

Table 3-4. Morphotype groupings from the bristle morphology and follicle anatomy data. Two k-means cluster analyses were conducted in Matlab (MATLAB and Statistics Toolbox Release 2019a, The MathWorks, Inc., Natick, Massachusetts, United States). The morphology variables included the discrete categories of bristle length, width, number and branching, and the anatomy variables included were the discrete categories of muscle bundle size, tissue density and Herbst corpuscle number. Both datasets were partitioned into five defined groups using k-means distance measures. The morphology grouping agreed with the phylogeny, but the follicle anatomy grouping did not, and varied from species to species.

Species	Bristle morphology morphotypes	Follicle anatomy Morphotypes
P. strigoides	Group 1	Group 1
B. auritus	Group 1	Group 1
B. stellatus	Group 1	Group 1
C. nacunda	Group 2	Group 3
C. minor	Group 2	Group 3
E. argus	Group 2	Group 4
C. pectoralis	Group 3	Group 2
C. vexillarius	Group 3	Group 3
C. europaeus	Group 3	Group 5
N. albicollis	Group 3	Group 1
A. cristatus	Group 5	Group 2
S. caripensis	Group 4	Group 2

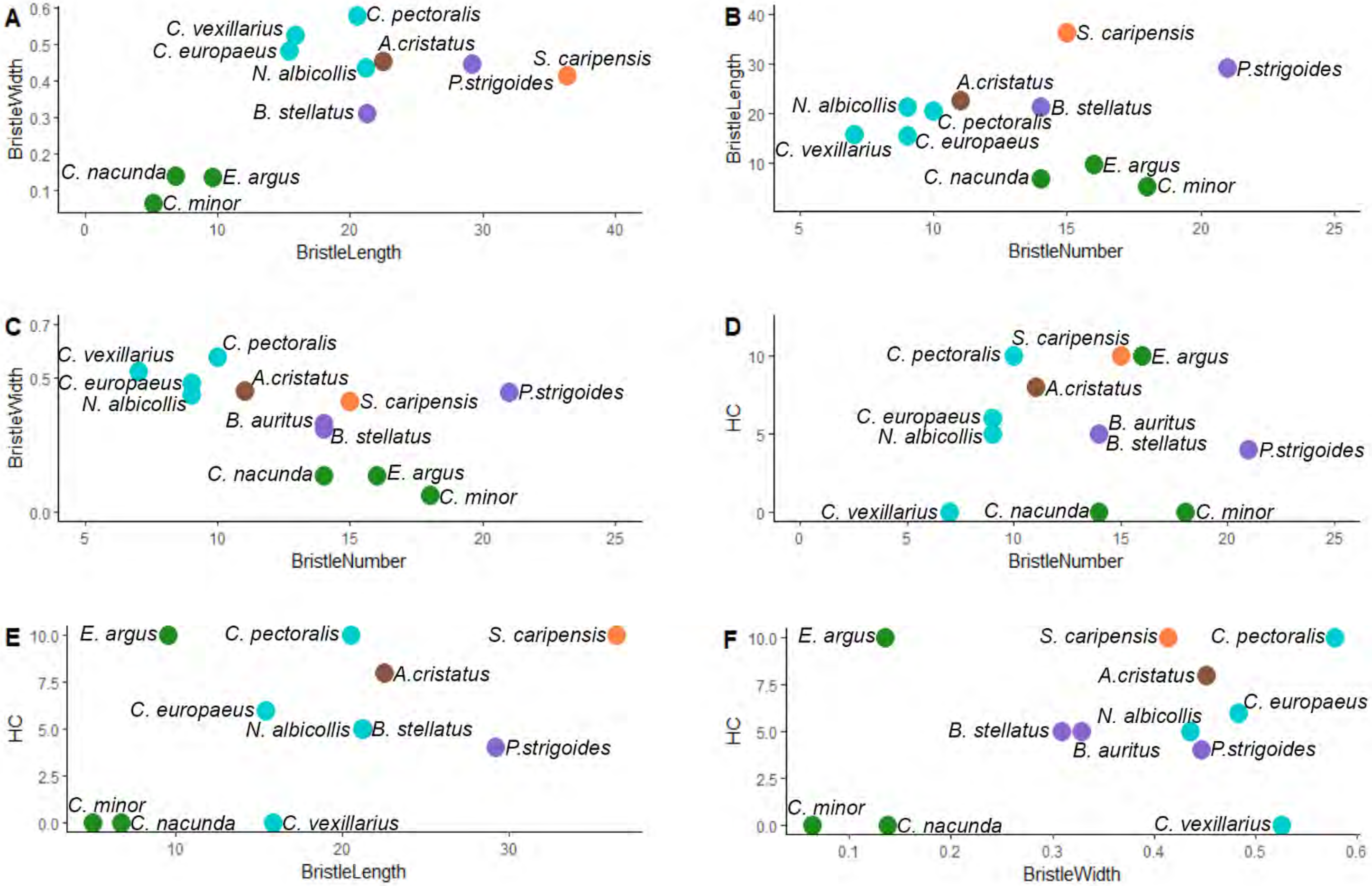


Figure 3-5. Scatterplots of A) Bristle length against Bristle width, **B)** Bristle number against Bristle length, **C)** Bristle number against Bristle width, **D)** Bristle number against Herbst corpuscle number, **E)** Bristle length against Herbst corpuscle number, and **F)** Bristle width against Herbst corpuscle number. Species are coloured coded following their bristle morphology: frogmouths in purple, nighthawks and spotted nightjar in green, nightjars and pauraque in blue, Australian owlet-nightjar in brown and oilbird in orange.

3.3.2 Follicle Anatomy

Characterisation of the follicle anatomy

Staining of the skin of the rictal region revealed high inter-species variability of the bristle-bearing integument, especially in the presence of Herbst corpuscle mechanoreceptors (Figure 3-6) and the intrinsic fibres in the surrounding of the bristle follicles (Table 3-5). There was no clear pattern in follicle anatomy morphotypes, based on a k-means cluster analysis (Table 3-4).

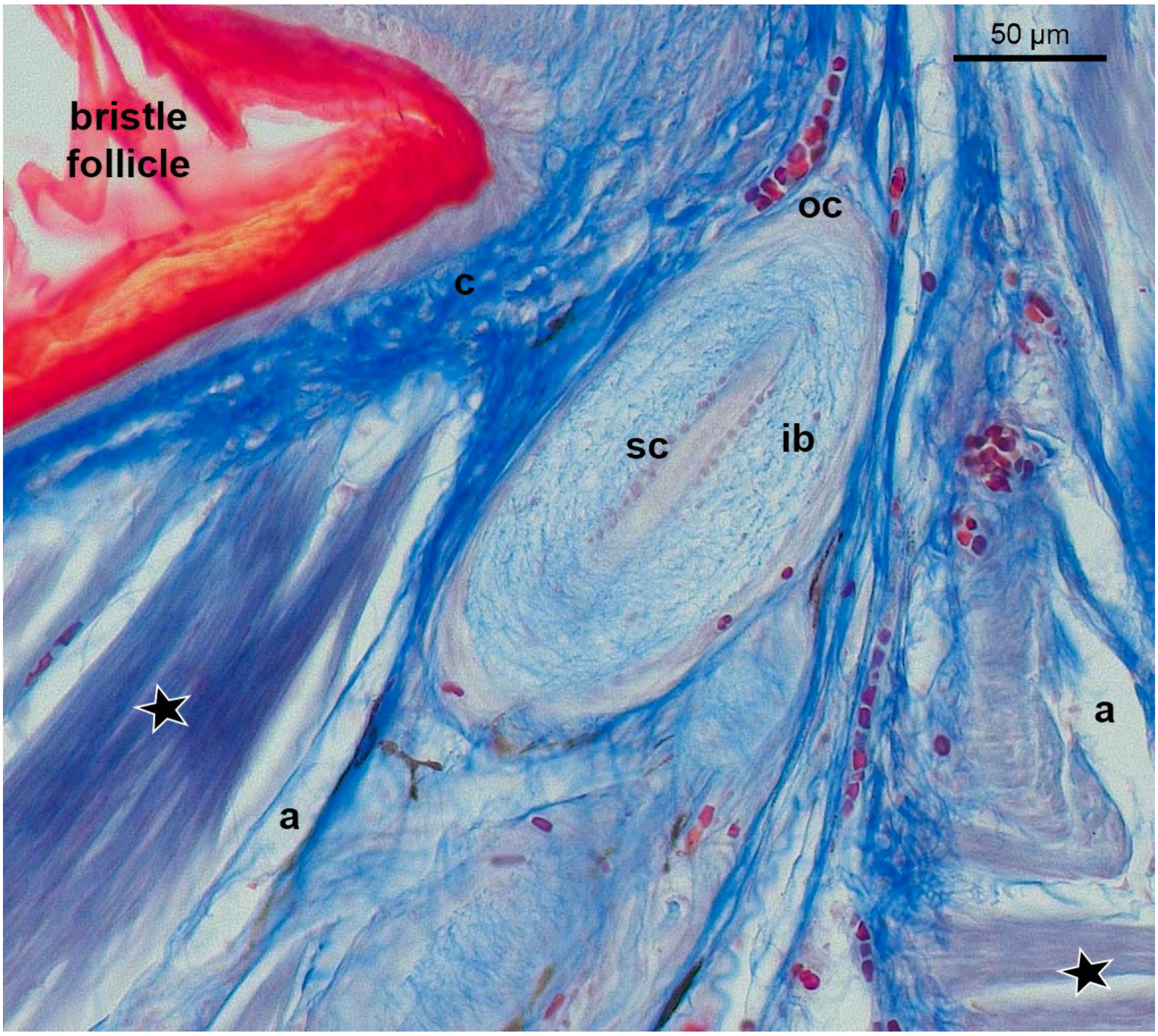


Figure 3-6. Longitudinal section of the dermis layer showing a cross-section of Herbst corpuscles around a bristle follicle from the skin region above the upper beak of *C. pectoralis*. Star: muscle fibres; **(a)** adipose tissue; **(c)** connective tissue; **(oc)** outer capsule; **(ib)** inner bulb; **(sc)** rows of Schwann cells. Sections stained with Masson's trichrome stain.

The bristle-bearing integument of *P. strigoides, B. auritus* and *B. stellatus* revealed a dense tissue (Figure 3-7, 8, 9; Table 3-5) with the smooth erector or depressor muscles running diagonally from consecutive bristle follicles. In *P. strigoides* and *B. auritus*, the smooth apterial bristle muscle was also present joining the distal tips of bristles together (Figure 3-7, 8). In all three specimens, the dermis contained connective tissue around the rictal bristle follicles (shown as a blue ring around the follicle; Figure 3-7, 8, 9). A few Herbst corpuscles mechanoreceptors were present in the surroundings of the rictal bristle follicles (Figure 3-7, 8, 9; Table 3-5). Herbst corpuscle structure possessed a characteristic outer capsule enclosing an onion-shaped lamellate outer zone and an inner bulb, surrounded by two rows of Schwann cell nuclei (Figure 3-6).

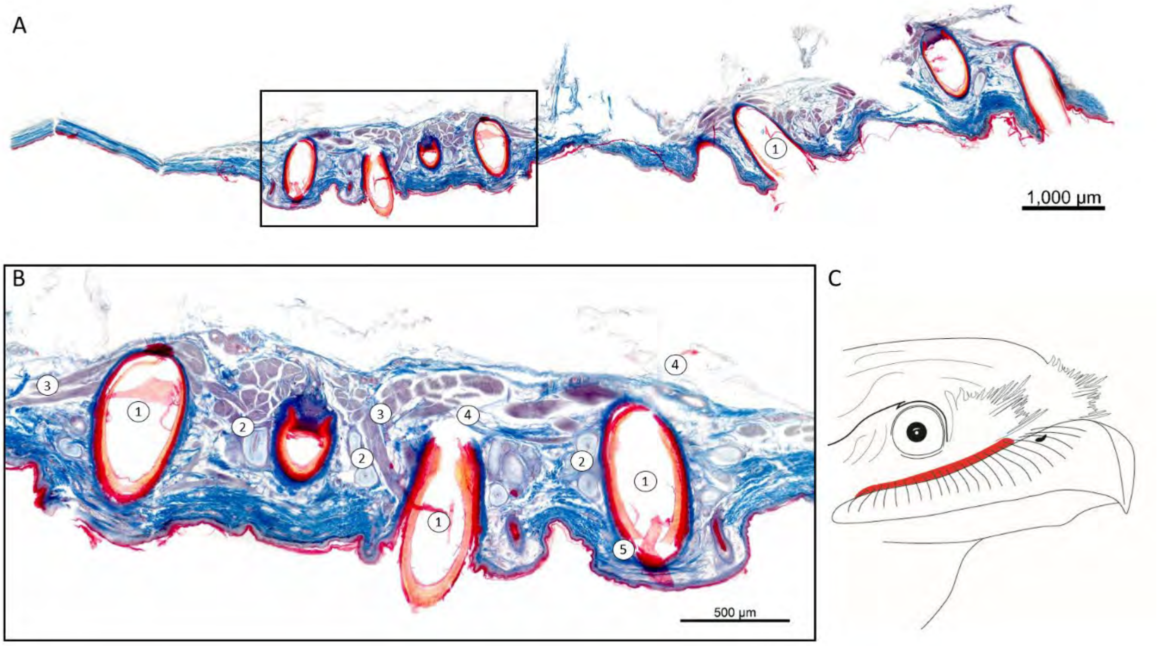


Figure 3-7. A) Longitudinal section of bristle follicles from the skin region above the upper beak of *Podargus strigoides*, from nare to rictus (right to left). **B)** Enlargement of the row of bristle follicles (**1**), which reveals the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), dermal muscle fibres (**3**), adipose tissue (**4**) and connective tissue (**5**) surrounding the follicles. Sections stained with Masson’s trichrome stain. **C)** Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *P. strigoides*.

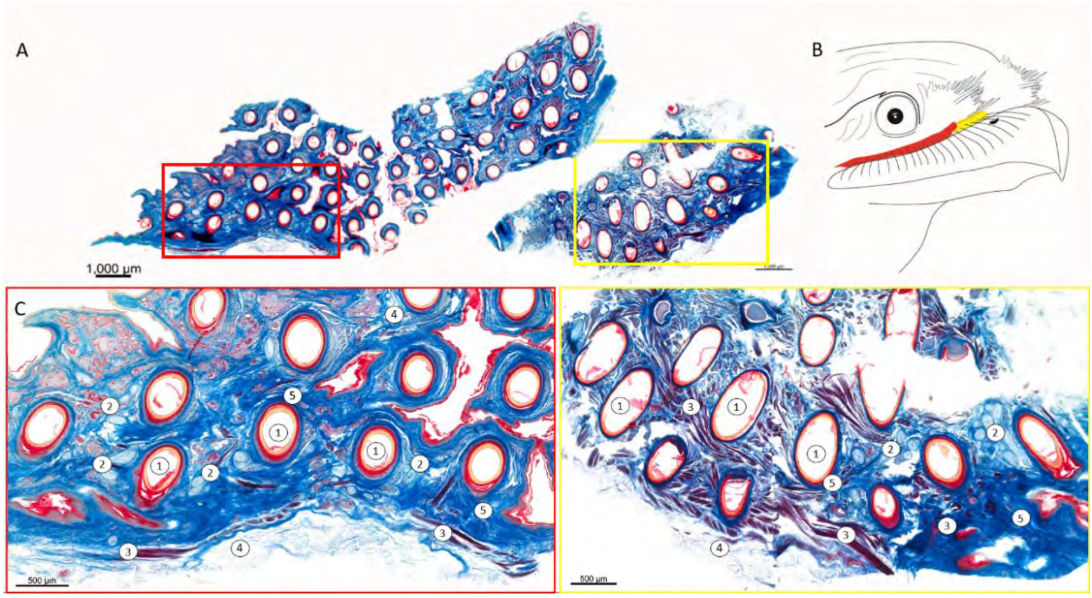


Figure 3-8. A) Longitudinal section of bristle follicles from two skin regions above the upper beak of *Batrachostomus auritus*, from nare to rictus (right to left). **B)** Schematic drawing illustrating in red and yellow correspondent skin regions cut along the upper beak, enclosing the bristle follicles of *B. auritus*. **C)** Enlargement of the row of bristle follicles (**1**) of *B. auritus* (red and yellow section), which reveals the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), dermal muscle fibres (**3**), adipose tissue (**4**) and connective tissue (**5**) surrounding the follicles. Sections stained with Masson's trichrome stain.

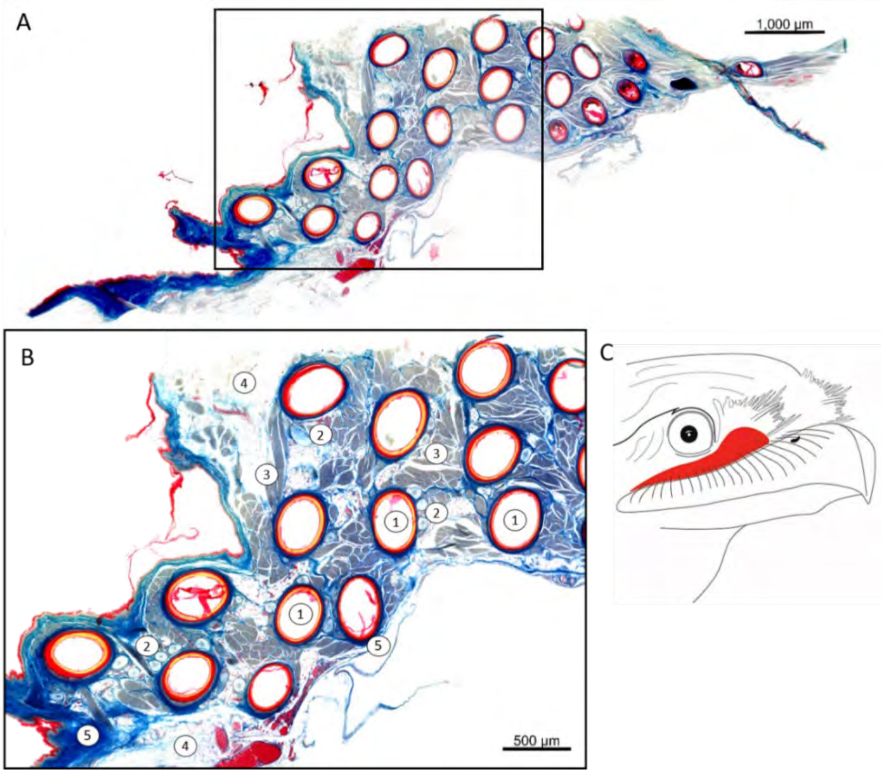


Figure 3-9. A) Longitudinal section of bristle follicles from the skin region above the upper beak of *Batrachostomus stellatus*, from nare to rictus (right to left). **B)** Enlargement of the row of bristle follicles (**1**), which reveals the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), dermal muscle fibres (**3**), adipose tissue (**4**) and connective tissue (**5**) surrounding the follicles. Sections stained with Masson's trichrome stain. **C)** Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *B. stellatus*.

The integument of *C. nacunda*, *C. minor* and *E. argus* revealed porous tissue within the dermis (Figure 3-10, 11, 12; Table 3-5). Samples from all three species had bristle follicles enclosed in connective tissue (Figure 3-10, 11, 12). Although, *C. nacunda* had no intrinsic fibres present in the surroundings of the bristle follicles (Figure 3-10), *C. minor* and *E. argus* had small bundles of the smooth erector or depressor muscle fibres extending from the base of a follicle to the neck of another one (Figure 3-11, 12; Table 3-5). There were no mechanoreceptors present in the tissue immediately surrounding the rictal bristles in *C. nacunda* or *C. minor* (Figure 3-10, 11), whereas a high number of Herbst corpuscles were found in the tissue surrounding the rictal bristle follicles in *E. argus* (Figure 3-12; Table 3-5).

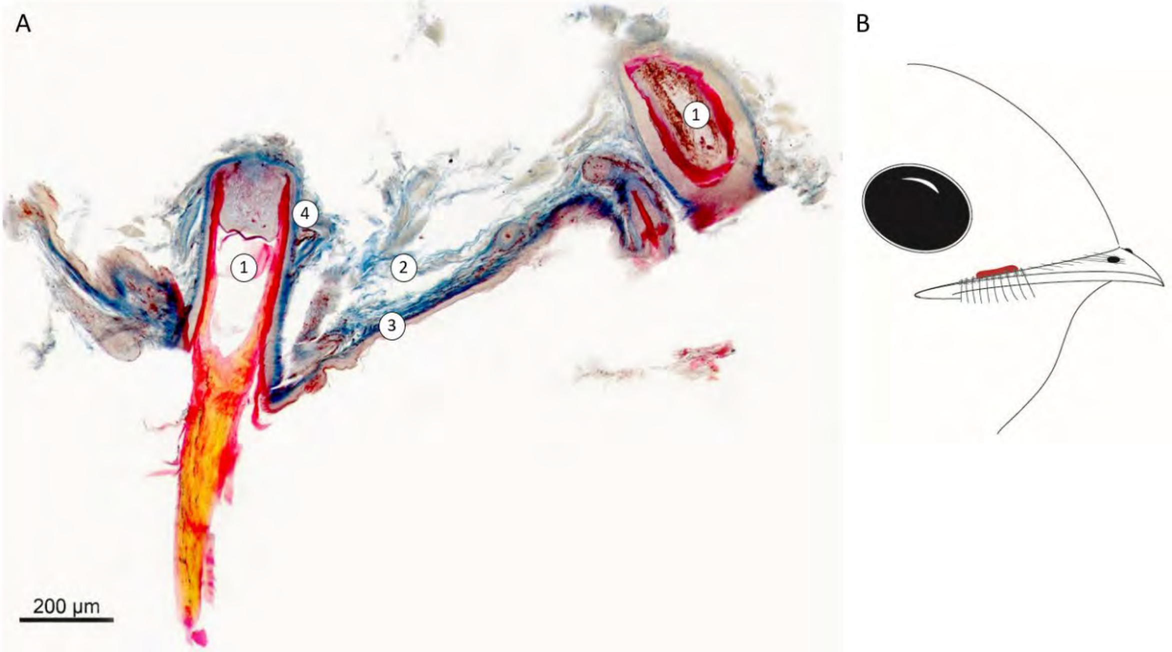


Figure 3-10. **A) Longitudinal section of bristle follicles from the skin region above the upper beak of *Chordeiles nacunda***, from the nare to rictus (right to left). The photo shows the dermis integument, delimited by the epidermis (**3**) containing bristle follicles (**1**) surrounded by adipose tissue (**2**) and connective tissue (**4**). Section stained with Masson’s trichrome stain. **B)** Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *C. nacunda*.

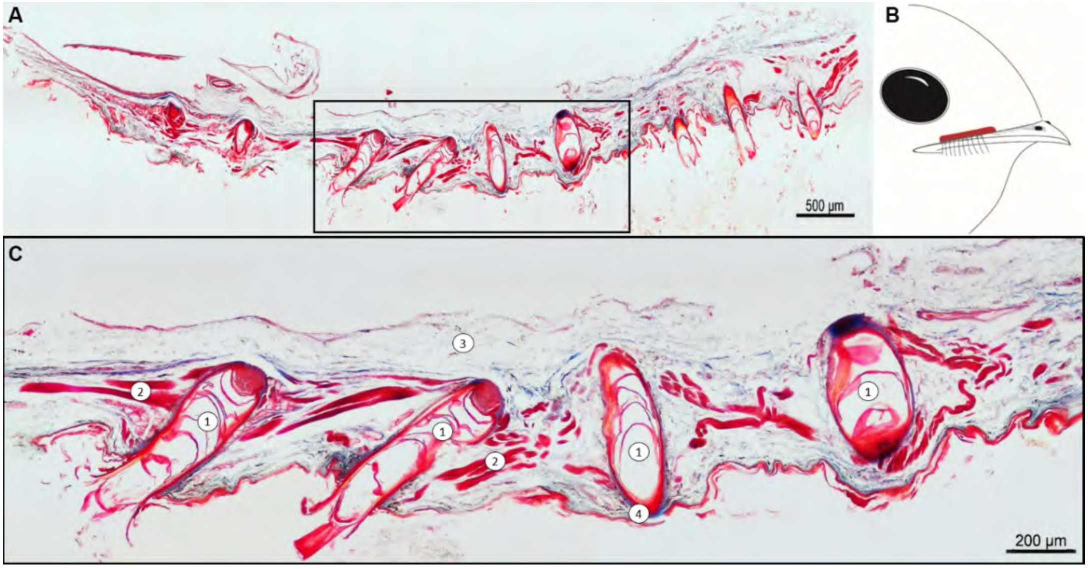


Figure 3-11. A) Longitudinal section of bristle follicles from the skin region above the upper beak of *Chordeiles minor*, from the nare to rictus (right to left). **B)** Enlargement of the row of bristle follicles (**1**), which reveals the presence of dermal muscle fibres (**2**), adipose tissue (**3**) and connective tissue (**4**) surrounding the follicles. **C)** Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *C. minor*. Sections stained with Masson's trichrome stain.

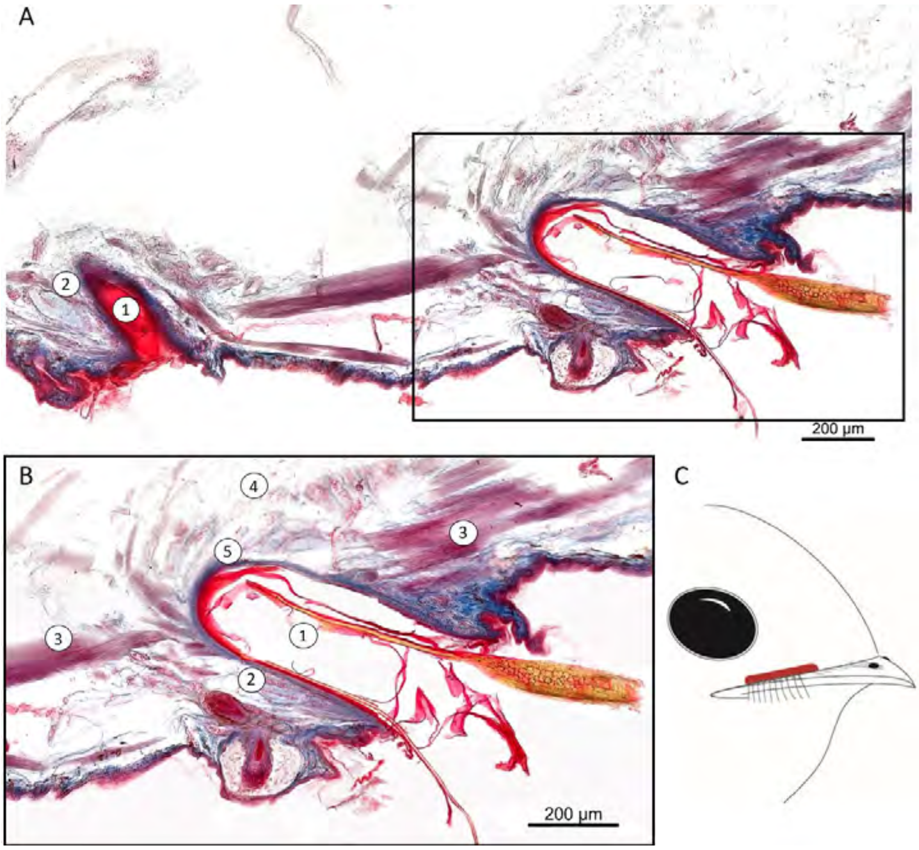


Figure 3-12. **A) Longitudinal section of bristle follicles from the skin region above the upper beak of *Eurostopodus argus***, from nare to rictus (right to left). **B)** Enlargement on a bristle follicle (**1**), which reveals the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), dermal muscle fibres (**2**), adipose tissue (**4**) and connective tissue (**5**) surrounding the follicles. Sections stained with Masson's trichrome stain. **C)** Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *E. argus*.

There was interspecific variation in the musculature, mechanoreceptors and density of the bristle-bearing integument in *C. pectoralis, C. vexillarius, C. europaeus* and *N. albicollis* (Figure 3-13 to 16). *C. pectoralis* displayed a dense tissue (Figure 3-13; Table 3-5), with a dermis presenting rictal bristle follicles connected together by a dense layer of smooth apterial muscle (Figure 3-13). A high number of mechanoreceptors were present all around the rictal bristle follicles in *C. pectoralis*, from the distal tip of the follicle (Figure 3-13; Table 3-5).

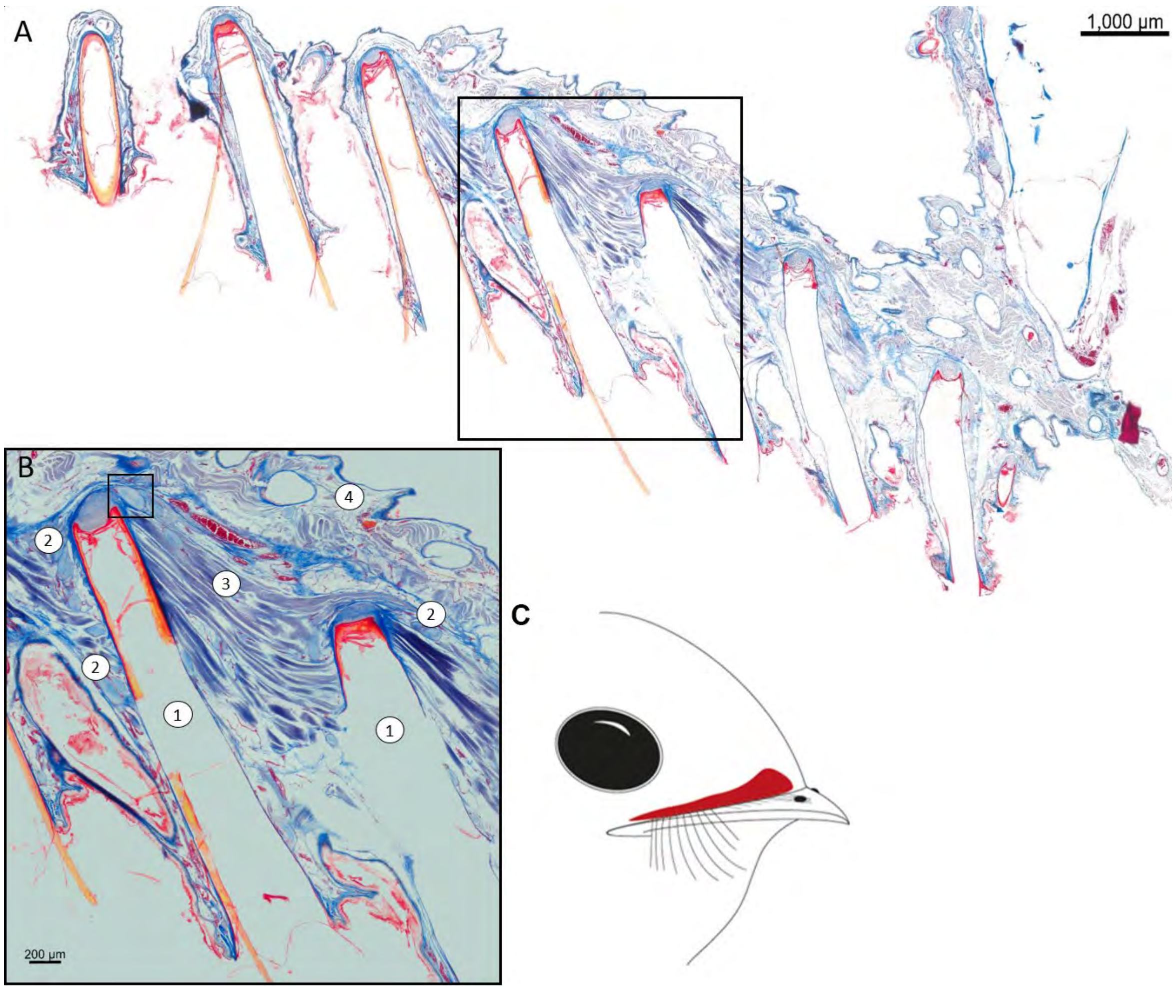


Figure 3-13. A) Longitudinal section of skin showing a cross-section of mature bristle follicles from the skin region above the upper beak of *Caprimulgus pectoralis*, from the nares to the rictus (right to left). **B)** Enlargement on two mature bristle follicles (**1**), which reveals the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), muscle fibres (**3**), and adipose tissue (**4**) surrounding the bristle follicles (**1**), and connective tissue (**5**) enveloping the follicle base in the dermis. Sections stained with Masson's trichrome stain. **C)** Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *C. pectoralis*.

The skin tissue of *C. vexillarius* revealed a porous integument, in which small bundles of smooth erector or depressor muscles fibres connected the bristle follicles together (Figure 3 -14; Table 3 -5). There was no evidence of mechanoreceptors in the bristle-bearing integument of *C. vexillarius* (Figure 3 -14; Table 3 -5).

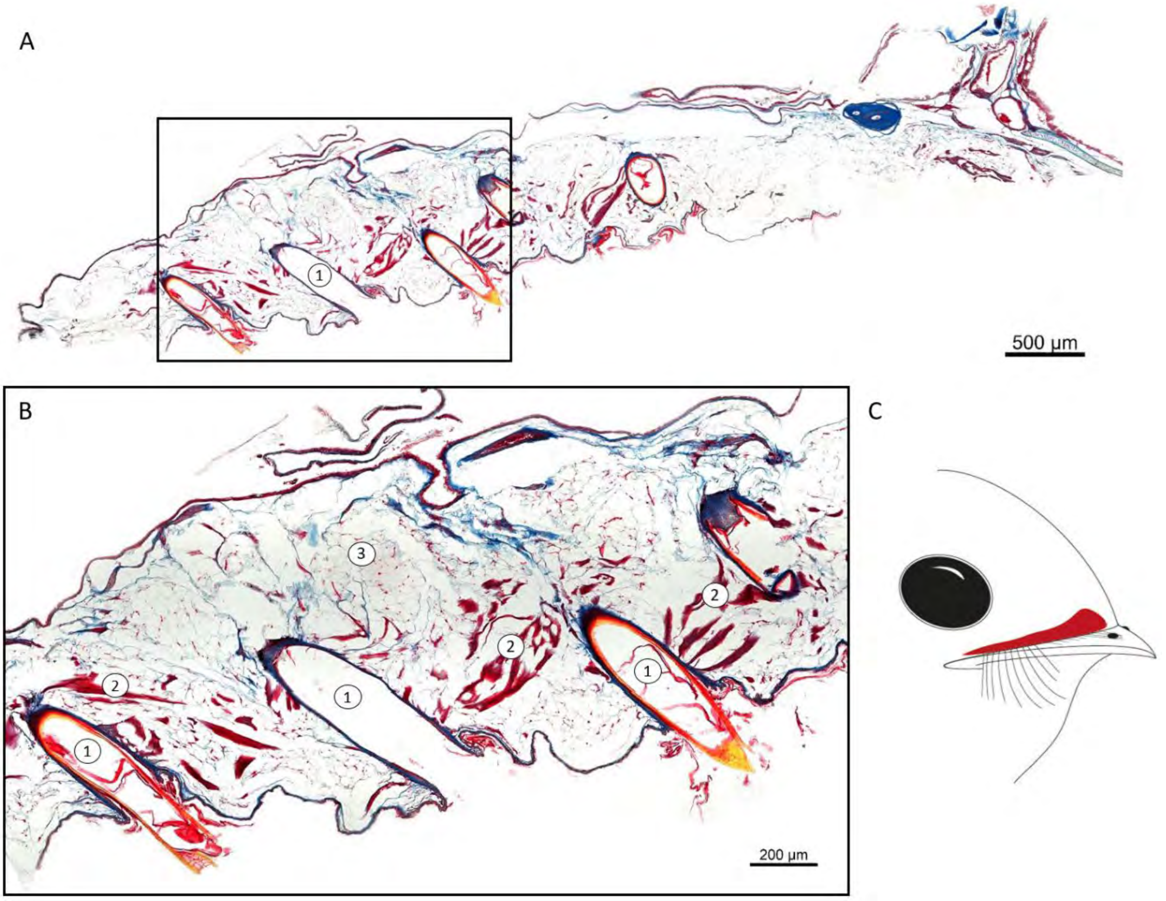


Figure 3-14. A) Longitudinal section of bristle follicles from the skin region above the upper beak of *Caprimulgus vexillarius*, from the nare to rictus (right to left). **B)** Enlargement on the row of bristle follicles (**1**), which reveals the presence of dermal muscle fibres (**2**) connecting the bristle follicles (**1**) together, in a dermis high in adipose tissue (**3**) concentration. Sections stained with Masson's trichrome stain. **C)** Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *C. vexillarius*.

The integument of *C. europaeus* consisted of a porous dermis with a small bundle of smooth erector or depressor muscle fibres that appeared to connect bristle follicles together, and a thin layer of connective tissue was apparent at the base of the bristle follicles (Figure 3 -15; Table 3 -5). In addition, Herbst corpuscles were present around the rictal bristle follicles in a small number and constricted at the base of the follicle (Figure 3 -15; Table 3 -5).

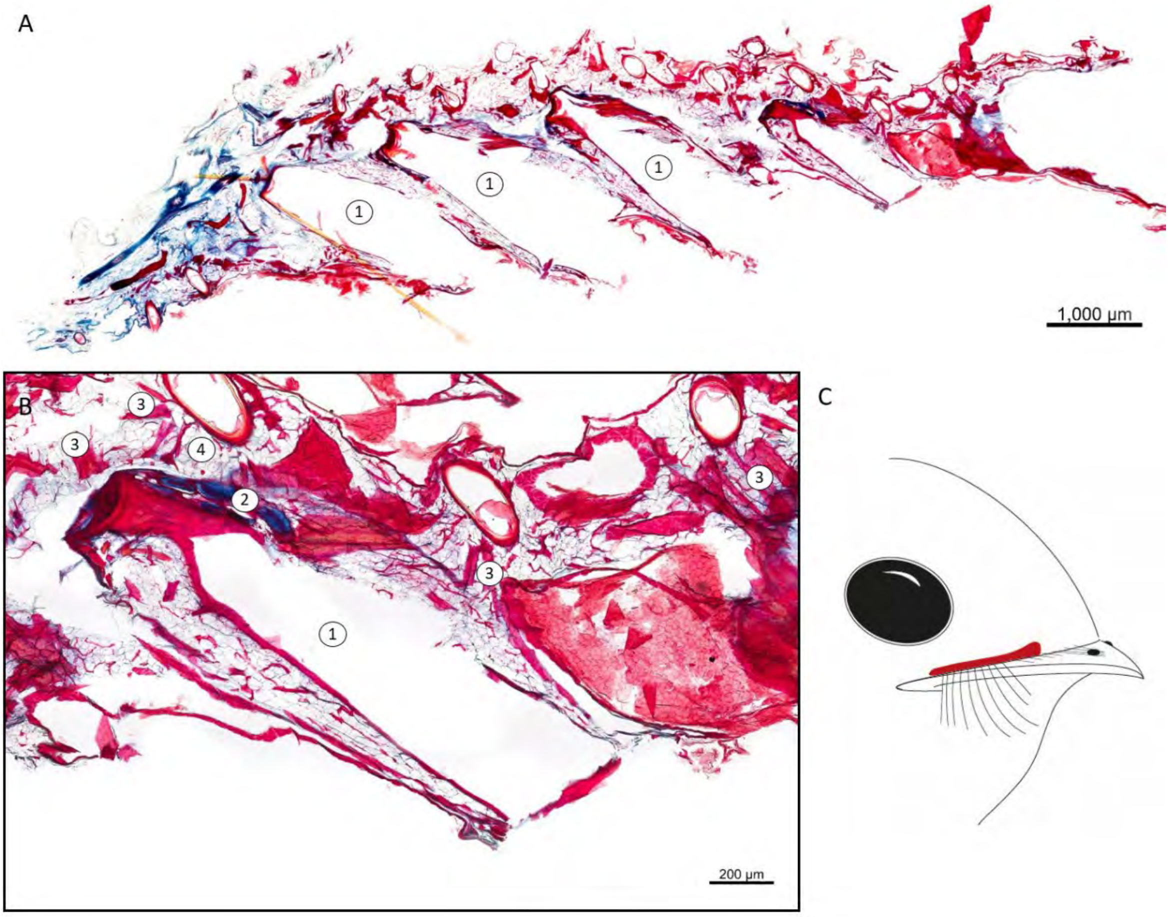


Figure 3-15. **A) Longitudinal section of bristle follicles from the skin region above the upper beak of *Caprimulgus europaeus***, from the nare to rictus (right to left). **B)** Enlargement on the row of bristle follicles (**1**), which reveals the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), dermal muscle fibres (**3**) and adipose tissue (**4**) surrounding the follicles. Sections stained with Masson's trichrome stain. **C)** Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *C. europaeus*.

The integument of *N. albicollis* revealed a dense tissue, and large bundle of smooth erector or depressor muscle fibres, pairing bristle follicles together (Figure 3 -16; Table 3 -5). The follicles were also surrounded by connective tissue, in which the muscle fibres seemed to be attached with. Herbst corpuscles were present all around the follicles, from the distal tip to the neck of the follicle (Figure 3 -16), however the number of mechanoreceptors in *N. albicollis* was low, compared to the other species (Figure 3 -16; Table 3 -5).

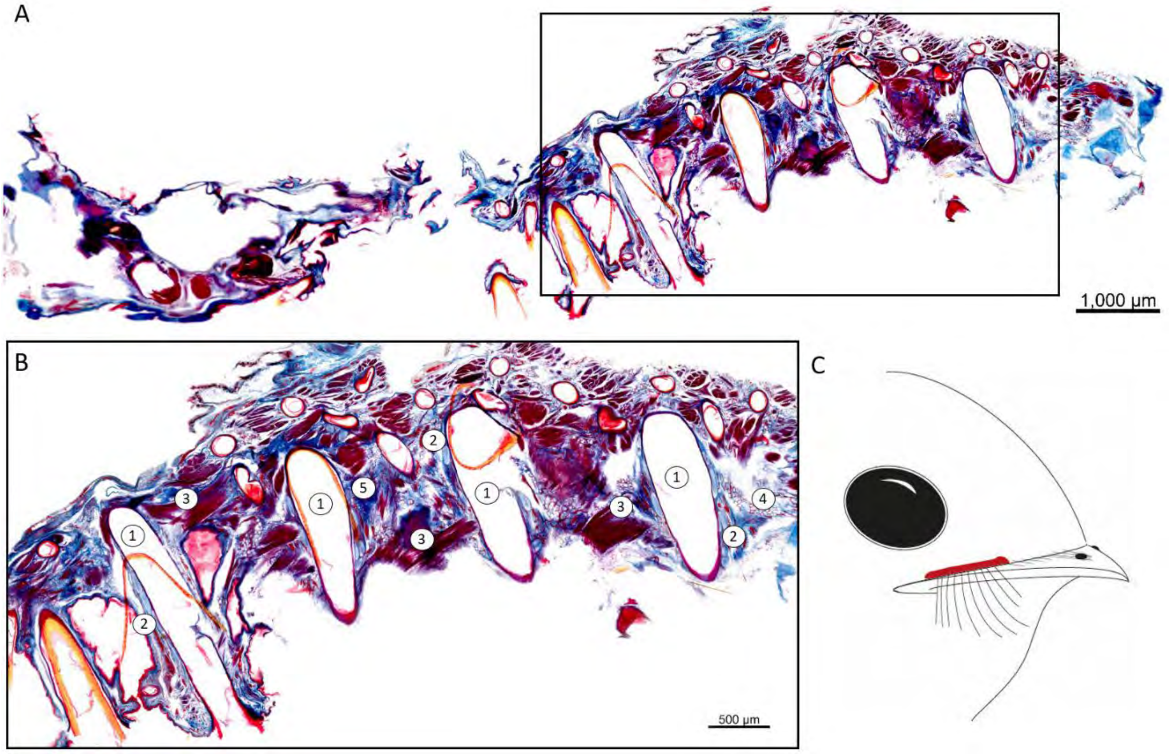


Figure 3-16. A) Longitudinal section of bristle follicles from the skin region above the upper beak of *Nyctidromus albicollis*, from the nare to rictus (right to left). **B)** Enlargement of the row of bristle follicles (**1**), which reveals the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), dermal muscle fibres (**3**), adipose tissue (**4**) and connective tissue (**5**) surrounding the follicles. Sections stained with Masson's trichrome stain. **C)** Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *N. albicollis*.

The sections from the *A. cristatus* specimen had a different orientation (angle) to the other samples and did not show the same longitudinal section of the bristle follicle. *A. cristatus* had a dense tissue (Figure 3 -17; Table 3 -5), with large bundles of the smooth erector or depressor muscle fibres, pairing the bristle follicles together. Furthermore, bristles follicle appeared to be surrounded by connective tissue and Herbst corpuscles (Figure 3 -17). Despite the different orientation (angle), a high number of Herbst corpuscles around the follicles were distinguishable (Figure 3 -17; Table 3 -5).

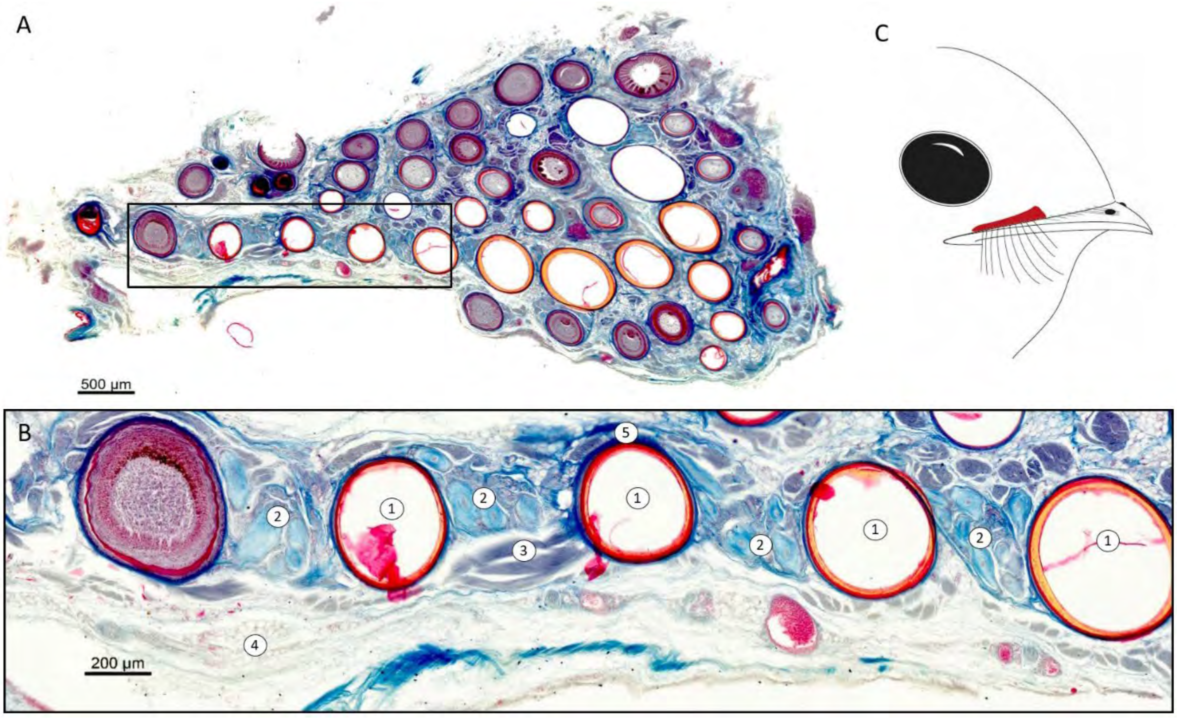


Figure 3-17. A) Cross-section of bristle and feather follicles from the skin region above the upper beak of *Aegotheles cristatus*, from nare to rictus (right to left). **B)** Enlargement of the row of bristle follicles (**1**), which reveals the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), intrinsic fibres (**3**), adipose tissue (**4**) and connective tissue (**5**) surrounding the follicles. Sections stained with Masson's trichrome stain. **C)** Schematic drawing illustrating in red the skin region cut along the upper beak, enclosing the bristle follicles of *A. cristatus*.

The integument of *S. caripensis* consisted of a dense tissue, with a large sheet of striated subcutaneous muscle fibres underlying the bristles follicles (Figure 3 -18; Table 3 -5). *S. caripensis* had abundant connective tissue around the follicle, as well as numerous Herbst corpuscles (Figure 3 -18; Table 3 -5).

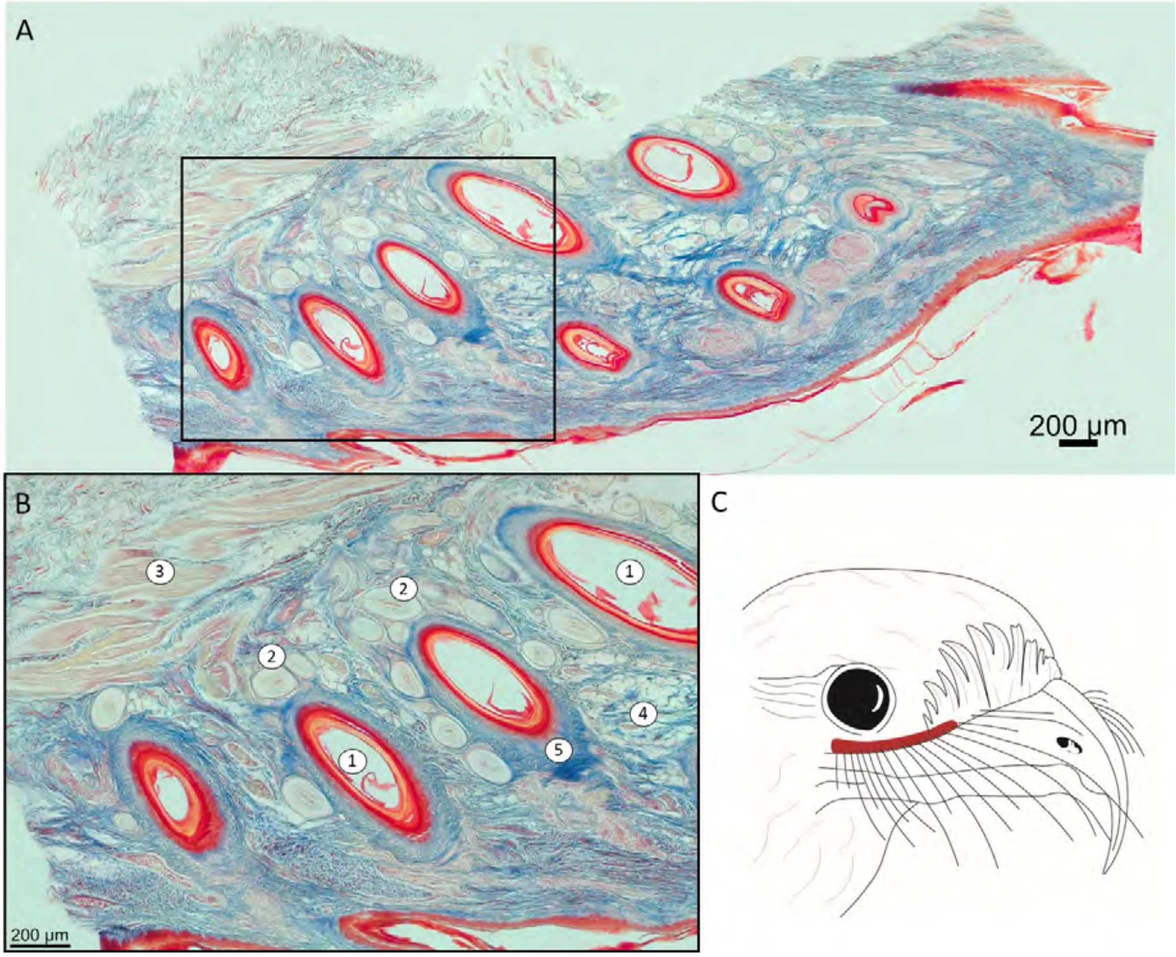


Figure 3-18. **A) Longitudinal section of bristle follicles from the skin region above the upper beak of *Steatornis caripensis***, from nare to rictus (right to left). **B)** Enlargement of the row of bristle follicles (**1**), which reveals the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), adipose tissue (**4**) and connective tissue (**5**) surrounding the follicles, and muscle fibres (**3**) underlying the bristle follicle tract. Sections stained with Masson's trichrome stain. **C)** Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *S. caripensis*.

Relationship between bristle morphology and follicle anatomy

Herbst corpuscle number was not significantly correlated with bristle length (N=11, Spearman's Rank: *rho*=0.39, *p*= 0.23) or bristle width (N=12, Spearman's Rank: *rho*=0.23, *p*= 0.47) (Figure 3-5). However, *S. caripensis,* which had the longest bristles, also had the highest number of mechanoreceptors, while the two *Chordeiles* species, which had the shortest and thinnest bristles, had no mechanoreceptors present (Table 3-5). While not able to be tested statistically, small bundles of muscle fibres tended to be present in species with short (*C. minor* and *E. argus*) and medium-sized bristles (*C. vexillarius* and *C. europaeus*), whereas large muscle bundles were present in species with medium or long bristles (Table 3-5). Herbst corpuscle number was not correlated to bristle number (N= 12, Spearman's Rank: *rho*= -0.03, *p*= 0.93; Figure 3-5). Therefore, although there are some anecdotal associations of bristle morphology and follicle anatomy in our data, quantitative measurements of bristle length, width and number were not correlated with the number of Herbst corpuscles.

Table 3-5. Summary table of the anatomical descriptions of the bristle-bearing integument of the 12 species of the Caprimulgiform order. HC is Herbst corpuscles. HC position corresponds to the position of Herbst corpuscles at the bristle follicle. Bristle number corresponds to the number of bristle on one side of the face. Species names colour-coded following their bristle morphotypes, based on bristle size (length and width), shape (branching present and absent) and number (not follicle anatomy): frogmouths in purple, nighthawks and spotted nightjar in green, nightjars and pauraque in blue, Australian owlet-nightjar in brown, and oilbird in orange.

Species	Adjacent muscle	Fibres bundle	Connective tissue	HC position	HC number	Dermis density	Quantity of adipose tissue	Bristle number
P. strigoides	smooth erector/depressor muscle smooth apterial muscle	Large	Yes	Around	4	Dense	20%	20.8 ± 1.5
B. auritus	smooth erector/depressor muscle smooth apterial muscle	Large	Yes	Around	5	Dense	10%	14 ± 1.4
B. stellatus	smooth erector/depressor muscle	Large	Yes	Around	5	Dense	30%	14 ± 0
C. nacunda	Absent	Absent	Yes	Absent	0	Porous	70%	13.5 ± 2.1
C. minor	smooth erector/depressor muscle	Small	Yes	Absent	0	Porous	70%	18 ± 0
E. argus	smooth erector/depressor muscle	Small	Yes	Around	10	Porous	60%	16± 2
C. pectoralis	Smooth apterial muscle	Large	Yes	Around	10	Dense	40%	10 ± 0
C. vexillarius	smooth erector/depressor muscle	Small	Yes	Absent	0	Porous	80%	6.5 ± 0.7
C. europaeus	smooth erector/depressor muscle	Small	Yes	Base	6	Porous	60%	9 ± 0
N. albicollis	smooth erector/depressor muscle	Large	Yes	Around	5	Dense	20%	8.8 ± 1.3
A. cristatus	smooth erector/depressor muscle	Large	Yes	Around	8	Dense	40%	11 ± 0
S. caripensis	striated subcutaneous muscle	Large	Yes	Around	10	Dense	10%	15 ± 1.4

3.4 Discussion

Bristle morphology

I identified five different bristle morphotypes in the twelve Caprimulgiformes species investigated in this study (Figure 3-4; Table 3-4), which varied in bristle size (length and width), shape (branching present and absent) and number. These morphotypes were in accordance with a contemporary Caprimulgiform phylogeny (Figure 3-4; Table 3-4), where closely related species had similar bristle morphologies. I also noticed that the beak and head shapes of the species affected the position of the bristles, and hence the dissection area (Figure 3-1). Beak shape is one of the most diverse traits in birds, varying in morphology according to individual species and their feeding strategies (Stettenheim, 2000; Cunningham et al., 2013; Thomas et al., 2016). Due to the small sample sizes and specimen availability of the species in this study, I am not able to conduct phylogenetic analyses; however, I demonstrate below that bristle morphology does not coincide consistently with beak shape and foraging traits, rather it is distributed non-randomly over the contemporary Caprimulgiform phylogeny.

Seven of our sampled species (*C. pectoralis, C. vexillarius, C. europaeus, N. albicollis*, *C. nacunda, C. minor* and *E. argus*) are predominantly insectivorous with small, thin beaks and a wide mouth gape (Cleere, 1998). However, while the aforementioned species share a similar beak shape and diet, they vary widely in their bristle morphology, including length, width and branching. Hence, *C. pectoralis, C. vexillarius, C. europaeus, N. albicollis* appear to be a distinct group from *C. nacunda, C. minor* and *E. argus*, both in terms of bristle morphology and phylogenetically. Although *A. cristatus* has the same diet as the seven previous species, it has a slightly more keratinised and hooked beak than the others (Cleere, 1998), and possesses a combination of unbranched and branched bristles. There are also examples in our specimens where a congruence seems to appear between bristle morphology, beak shape, foraging traits and phylogeny. *P. strigoides, B. auritus* and *B. stellatus*, all shared similar beak shape, diet (Cleere, 1998) and bristle morphology, and so they form a sister group, as these features differed from our other species (Figure 3-4). *S. caripensis* has a different beak shape, diet (Cleere, 1998) and bristle morphology than all the other species (Figure 3-4), consequently *S. caripensis* is rather distinct from the others and is on its own within the phylogeny. Measuring the bristle morphology of more specimens and species will allow us to explore these relationships further, especially by using phylogenetic analyses.

Follicle anatomy

For all species examined in this study, I found that bristle follicles were anchored in the dermis of the skin with an outermost layer of connective tissue, which is consistent with previous studies on avian rictal bristles (Ostmann et al., 1963; Homberger and De Silva, 2000). The appearance of muscle fibres connecting the follicles has also been observed in contour feathers, such as in the wild turkey (*Meleagris gallopavo*) (Homberger and De Silva 2000; 2003). The size and arrangement of smooth muscles at the follicles have been found to vary between feather types, i.e. large flight feathers have larger muscle bundles than small filoplumes that have no muscles (Stettenheim, 2000). In agreement with this latter study, I also found that short, thin bristles had smaller muscle bundles than medium or long, wide bristles (Figure 3-4; Table 3-5).

Bristle morphology was not associated with follicle anatomy in the species in this study (Figure 3-7 to 18), since there was no correlation between the presence of muscles and the number of Herbst corpuscles, with bristle length, width or number. In mammalian whiskers, whisker length, width and number are associated with aspects of follicle anatomy and musculature (Muchlinski, 2010; Muchlinski et al., 2013; Grant et al., 2017). Specifically, that longer and more numerous whiskers tend to be associated with more arranged follicles and large intrinsic muscles (Muchlinski, 2010; Muchlinski et al., 2013; Grant et al., 2017), and that longer, thicker and stiffer whiskers tend to have numerous mechanoreceptors (Ebara *et al.*, 2002). However, this was not the case in our species. For example, *C. pectoralis, C. vexillarius* and *C. europaeus* had differences in follicle anatomy from one another, in terms of the size of muscle fibre bundles and mechanoreceptors, although they had the same bristle morphology. While bristle morphology visually agreed with the contemporary Caprimulgiform phylogeny (Figure 3-3, 4), the follicle anatomy (musculature and Herbst corpuscle number) did not. If this is the case, bristle follicle anatomy, and hence tactile sensitivity may be better explained by life-history traits, rather than phylogenetic relatedness. This has not been explored across a comparative data set before, and should form the basis of future studies on bristle anatomy and morphology.

Foraging traits and bristle function

The Caprimulgiform order is ecologically and behaviourally diverse, with species exhibiting different activity patterns, diet, foraging methods, foraging niches and patterns of habitat selection (Table 3-3) (Cleere, 1998). I suggest below that period of activity, foraging habitat selection and foraging method might all be associated with follicle anatomy in the Caprimulgiformes species that I studied here. Specifically, that tactile sensitive rictal bristles (with mechanoreceptors) are present in the Caprimulgiform species that forage in scotopic conditions in more closed, structurally complex habitats. Whereas bristles without mechanoreceptors are present in our Caprimulgiformes species that are open habitat, nocturnal cathemeral foragers.

Three of our study species that forage pre-dusk (*C. nacunda, C. minor* and *C. vexillarius*) had no mechanoreceptors present around their rictal bristles follicles, and had only short to medium length bristles (Figure 3-10, 11, 14; Table 3-4). The anatomy morphotype k-means cluster analysis also grouped these species together (Table 3-4). Although, *C. vexillarius* is found in semi-open habitats, whereas *C. nacunda* and *C. minor* are found in open habitats, all three species forage by hawking high above the canopy (in the case of *C. vexillarius*) or in open country or riverbeds (in the case of *C. nacunda* and *C. minor*) (Table 3-3; Holyoak, 2001). Therefore, nocturnal cathemeral species foraging in open habitat seem to have reduced bristle tactile function. The lack of mechanoreceptors suggests that these species may rely more on vision than touch, as flying during daylight in open habitats probably makes them less likely to collide with obstacles and more likely to visually detect prey. Rictal bristles in these species may still play a role in eye protection against flying items during feeding, despite them being less than 20 mm in length (Lederer, 1972; Conover and Miller, 1980). In addition, the bristles may also still be sensitive, as their movement could be detected in the absence of Herbst corpuscles by other mechanoreceptors, such as by stretch receptors like Ruffini corpuscles, that detect pressure and tension and are present in the muscle and skin (Halata and Munger, 1980).

Contrary to these nocturnal cathemeral species, I found that all nine obligate crepuscular and nocturnal species had mechanoreceptors present around their bristle follicles, and so are likely to have a tactile function. These species all forage in complex or closed habitats, which suggests that bristles may be involved in foraging, navigation and collision avoidance in the dark. The evolution of avian foraging traits often depends upon a trade-off between vision and other sensory systems (Cunningham et al., 2013; Corfield et al., 2015), therefore, touch sensing might well be more developed in nocturnally foraging species, as is the case in mammals (Muchlinski, 2010; Muchlinski *et al.*, 2013). The number of mechanoreceptors located around the follicle varied across our species, and I observed no clear relationship between the number of Herbst corpuscles and the anatomy morphotypes, with their foraging traits - the status of obligate crepuscular and nocturnal, and the density of their habitat (Table 3-3). This indicates that the sensitivity of rictal bristles is likely to be dependent upon a combination of species-specific life-history traits. For example, two obligate nocturnal species *B. auritus* and *B. stellatus* that prefer close habitats such as densely vegetated forest habitats, had low numbers of mechanoreceptors, while near-obligate nocturnal species *C. pectoralis, S. caripensis* and *E. argus*, had the highest number of Herbst corpuscles present, and live in both semi-open and closed habitat. The near-obligate nocturnal *C. europaeus* that prefers semi-open habitat (i.e. a mixture of open country and woodlands, Table 3-3), had low numbers of mechanoreceptors present at the base of the bristle follicle (Table 3-3). *C. europaeus,* like the nocturnal cathemeral species that lacked mechanoreceptors, is also an aerial hawking species (Cleere, 1998). Therefore, sensitive, tactile rictal bristles may not be beneficial for species that predominantly feed by aerial hawking.

Feathers have been found to guide navigation and foraging in a number of species, previously. For example, the crest and super-orbital plumes in whiskered auklets (*Aethia pygmaea*), are used to detect obstacles and guide navigation in dark crevices (Seneviratne and Jones, 2008). Brown kiwi (*Apteryx mantelli*) are nocturnal birds that have long rictal bristles, with a mean of 36.63mm (SD 7.97, n= 1 individual, 6 bristles) (Delaunay, unpublished data), which are longer than any of the bristles in our study species, and they also possess numerous (>10) Herbst corpuscle mechanoreceptors positioned all around the follicles (Fig. 3 in Cunningham *et al.*, 2011), which is similar in number to *E. argus*, *C. pectoralis* and *S. Caripensis* in our study (Figure 3-12, 13, 18). Cunningham *et al.*, (2011) suggest that, as Kiwis probe-forage holding their bristle forwards, they may use their tactually-sensitive rictal bristles to detect subterranean prey or to assess the quality of the foraging ground. Therefore, rictal bristles of *E. argus, C. pectoralis* and *S. caripensis* may be functionally similar and help to detect flying prey or obstacles during flight. Corfield *et al.* (2014) also proposed that brown kiwi rictal bristles are tactile and might help with navigation in the dark.

Conclusion

Rictal bristle morphology and follicle anatomy varied across the Caprimulgiform species of this study. Rictal bristle variation included the presence and number of mechanoreceptors in the immediate surroundings of the bristle follicles. Furthermore, species with short, thin, branching bristles lacked mechanoreceptors and tended to forage pre-dusk in open habitats; whereas species with mechanoreceptors around their bristle follicles tended to forage at night and in more closed habitats. Therefore, rictal bristles are likely to be tactile in many species, especially species foraging in lower light conditions. Further work investigating more species and specimens across the Caprimulgiformes and other avian orders will help to develop our understanding of bristle function, specifically addressing which ecological variables might be associated with the presence of long, sensitive bristles.

Chapter 4

Development of rictal bristles in *Podargus strigoides* and *Tyto alba*

Chapter summary:

Rictal bristles are likely tactile sensors, and tactile mechanoreceptors have been found within the rictal bristle follicles of some species, notably in *Podargus strigoides* and some owls. However, it is not known how rictal bristles develop, especially alongside a chick's normal developmental schedule. This study investigates the chick development schedule (body feather, rictal bristles, and eyes) and rictal bristle emergence of two species, *P. strigoides* and *T. alba.* It will describe behavioural changes during development that coincide with rictal bristle stimulation, and will identify the presence of mechanoreceptors in adults. Results demonstrated that both species are born without rictal bristles; both species are altricial and develop in four plumage stages. *P. strigoides* develop faster overall, yet their rictal bristles develop slower than *T. alba*'s. Both species have Herbst corpuscles around the rictal bristles follicles as adults, suggesting that adult rictal bristles are touch sensitive. Tactile sensitivity of the rictal region can also be observed in the chicks, by the gradual appearance of beak clapping, feeding behaviours (mouth movements, turning beak towards cotton bud) and avoidance following rictal region touches, as the chicks get older. While rictal bristles are unlikely to be functional in young chicks, their emergence and heightened behavioural responses coincide with independent feeding, suggesting that rictal bristles may play a role in feeding in older chicks and adults.

4.1 Introduction

The development of behaviour, physiology and anatomy of hatchling chicks varies across species, and depends upon the species, level of parental care and the environment (Starck and Ricklefs, 1998). Indeed, the level of parental care varies along a gradient of chick development types, from super-precocial to altricial (Starck and Ricklefs, 1998). Altricial chicks hatch in an underdeveloped state in which they are not capable of leaving the nest and feeding by themselves; therefore, they require a high level of parental care over a long period (Starck and Ricklefs, 1998; Gill, 2007). During their development, chicks will go through different plumage stages before fledging - from hatchling to young juvenile to complete juvenile/immature. Their plumage will consist entirely of down feathers, which will be superseded by juvenile feathers that will, in turn, be replaced by adult feathers (Bellairs and Osmond, 2005). Although feathers develop after hatching, feather follicles and the patterning of the feather tracts are formed at the embryonic stage and persist throughout the bird's life (Lucas and Stettenheim, 1972). However, the presence of mechanoreceptors (i.e. Herbst corpuscles) in the immediate surroundings of feather follicles varies according to the chick development type. In precocial chicks, mechanoreceptors are formed during embryonic development and are fully developed before hatching, whereas in altricial species the development of mechanoreceptors only starts after hatching (Páč and Malinovský, 1988). Evidence of tactile corpuscles present in the immediate surroundings of rictal bristle follicles show that rictal bristles are likely to be tactile (Chapter 3; (Cunningham et al., 2011; Delaunay et al., 2020); however, the development of the rictal bristle and the development of the tactile sensitivity of the rictal region have yet to be described.

Two species will be studied here, the tawny frogmouth (*Podargus strigoides*) and barn owl (*Tyto alba*). These species were selected due to their ease of access - at the zoo and in a breeding box project, respectively. They were also chosen due to the prominence of their rictal bristles and the likely presence of mechanoreceptors at their follicles. *P. strigoides* are often seen at night in Australia. However, its ecology, nesting behaviour and developmental schedule are still not extensively documented due to it being nocturnal, as well as cryptic and elusive (Körtner and Geiser, 1999a). Nesting behaviour and chick development is better described in *T. alba*, although previous studies have primarily focused on chick growth rate, body mass and diet (Haresign and Moiseff, 1988; Durant and Handrich, 1998; Durant et al., 2008), rather than the development of their feathers, especially rictal bristles. Mechanoreceptors have been found in the immediate surroundings of the rictal bristle follicles of the adult *P. strigoides* (Chapter 3), indicating a possible tactile sensitivity. Küster (1905) found mechanoreceptors in the surroundings of rictal bristle follicles in some owl species (*Bubo bubo, Asio flammeus, Athene noctua, Strix aluco*), indicating that they are likely to be vibrotactile sensors, but this work has not yet been done in *T. alba* yet.

This study will address the following hypothesis outlined in Chapter 2:

- **Rictal bristles will be present in hatchlings and play a role in the developing young**

Chicks have mechanoreceptors that are fully developed from birth but they undergo different plumage throughout their development, and the development of the feather is species-specific. Mammalian whiskers are essential in the early development of the pups, playing a role in navigation, huddling and feeding. Therefore, rictal bristles may also play an important role in the development of the chick. Rictal bristles are expected to develop relatively early in chick morphogenesis and be involved in feeding behaviour.

I will test both *T. alba* and *P. strigoides* as they differ in nesting ecology and diet, to also examine if any observed differences in rictal bristles emergence are associated with the development schedule of the chicks. Therefore, this study describes the developmental schedule of *P. strigoides* and *T. alba* chicks, particularly documenting the development of the feathers, body, eye opening and rictal bristle emergence. Rictal bristle follicle anatomy of adult *T. alba* will be studied to assess the possible presence of mechanoreceptors. Finally, to investigate if, and when, the rictal region becomes touch-sensitive, the chicks' behavioural responses to touch will be analysed in both species from birth to day 55-60.

4.2 Material and methods

4.2.1 Animals

Tawny frogmouth (*P. strigoides*) was chosen for this study since the adults have prominent rictal bristles, which have Herbst corpuscles within the immediate surroundings of their follicles (cf. Chapter 3), and are possibly altricial (Thompson, 1974). *P. strigoides* is also one of the largest Caprimulgiformae (Körtner and Geiser, 1999b) and, unlike other Caprimulgiformae species, can be found in zoos. The Woodland Park Zoo in Seattle, USA, keep breeding couples, which have chicks every year. Therefore, the *P. strigoides* chicks were captive and hand-reared. Data collection took place over a period of three years (2017 to 2019). In 2017, the zoo had four chicks, of which three completed their development attaining fledgling stage (49-52 days old), and one died after 7 days. In 2018, four chicks hatched, of which one died after 2 days, one after 4 days, one after 21 days, and no data was recorded after the fourth chick reached 30 days old. In 2019, only one chick was recorded and attained the fledgling stage (45 days). Therefore, in total, nine *P. strigoides* chicks were included in this study over three years, with data from four to six individuals each day (Table 4-1).

Barn owls (*T. alba*) were selected as they are an altricial species and possess prominent rictal bristles. Wild *T. alba* individuals were studied from May to June 2018 in the Hula Valley, Israel as part of *The Barn Owl Project*. Seven boxes had breeding pairs laying eggs that season. However, they did not all survive. Two boxes with a clutch of two and a clutch of eight survived, and the chicks were observed until the oldest reached day 53 and 58, and day 52 and 43 for the youngest, in their respective box. Clutches in the five other boxes did not survive, chicks died in different orders and at different ages. The experiment was still carried on with the surviving chicks of the day. In total, 39 *T. alba* chicks were filmed across the different boxes, with 2 - 8 individuals filmed per day (Table 4-1). In order to reduce disturbance, data collection of *T. alba* chicks occurred every 2 to 5 days, rather than daily as in *P. strigoides*. *T. alba* chicks were included in the study, once the oldest chick of the clutch had reached 14 days old, which coincided with the period where the parents stopped roosting in the box during the day and moved to a tree nearby, only returning to feed the chick at the night. *T. alba* chicks did not all hatch the same day, but every other day from each other, which enabled the behavioural response to be filmed in chicks younger than 14 days old. Sample numbers for both species, at each age, can be seen in Table 4-1.

4.2.2 Experimental procedures

P. strigoides and *T. alba* chick’s development of feathers, eye-opening and rictal bristle emergence were investigated by observing and comparing the individuals from video footage. Their behavioural responses to touch were also examined using the same footage. Although the exact set-up, and sample numbers differed between species, the experimental protocol was consistent (Figure 4-1). An individual chick was touched using a cotton bud, with pause of a couple of seconds between subsequent touches, on the top of the head, rictal region (as per Chapter 3), abdomen area and proximal region (the skin region directly above the beak and between the eyes, central to the face) (Figure 4-2), which were repeated three times for each chick per day. Total disturbance was less than a minute for *P. strigoides* per day and less than a minute per chicks of a box, every 2 to 5 days for *T. alba*. The top of the head and abdomen points were control points. The abdomen region was added as a negative control to the top of the head, as there was a possibility that the top of the head could still be a trigger point touched by parents when soliciting chicks to deliver food to their beak.

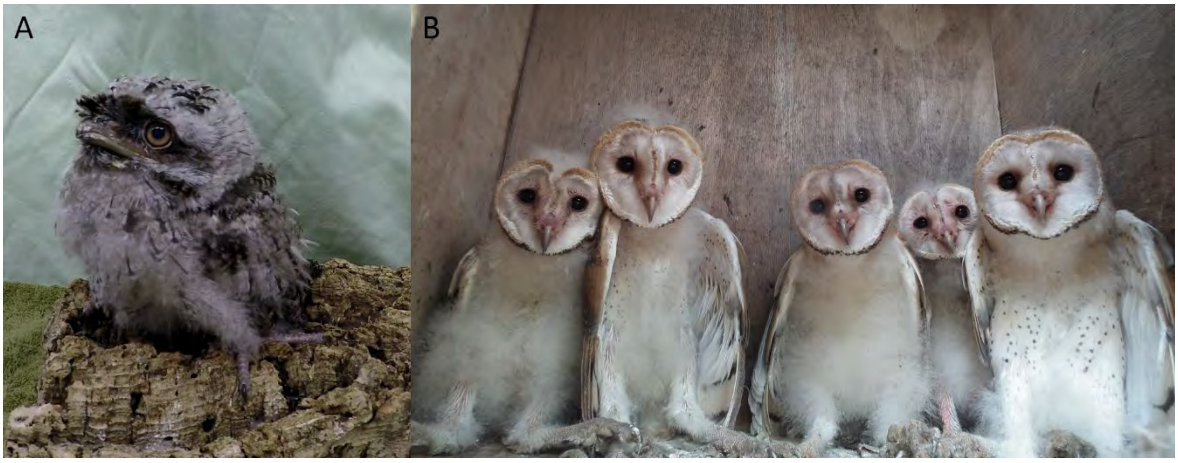


Figure 4-11. Photograph illustrating the set up for **A)** *Podargus strigoides* (one chick, day 24) on a fake log, and **B)** *Tyto alba* chicks (five owlets age from day 38 to 47) in their box.

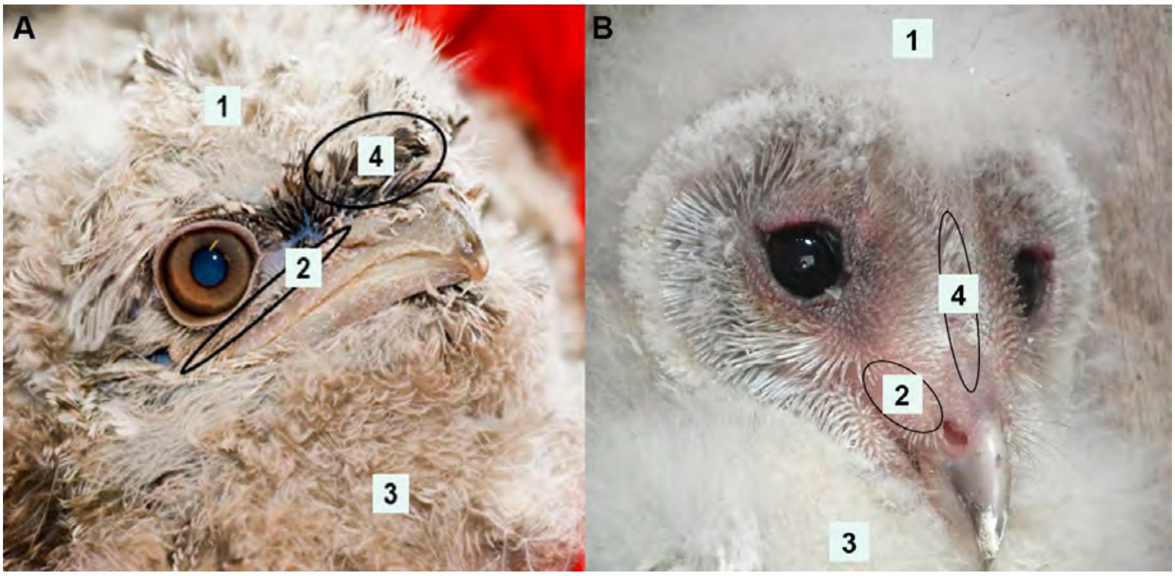


Figure 4-22. Photograph of A) *Podargus strigoides* (day 20) and B) *Tyto alba* (day 27) chicks with the four different touch point positions where the chick was repeatedly touched with a cotton bud during its development: 1) top of the head, 2) rictal region, 3) abdomen area, 4) Proximal region**.**

Table 4-1. Total number of individual filmed per species for each day of development and total number of individual filmed per species.

Day Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
P. strigoides	1	5	6	7	7	7	6	6	6	5	5	5	5	5	6
T. alba	0	4	2	1	2	1	3	3	2	2	2	4	2	5	5
Day	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
P. strigoides	6	6	6	6	5	6	5	5	5	5	5	4	5	5	5
T. alba	2	5	4	6	4	5	3	8	2	5	4	9	8	7	7
Day	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
P. strigoides	3	4	4	4	4	4	3	4	4	3	3	3	3	3	3
T. alba	7	7	6	8	10	9	5	13	8	10	5	10	5	2	8
Day	46	47	48	49	50	51	52	53	54	55	56	57	58	Total chicks filmed	
P. strigoides	2	2	2	3	2	1	1	0	0	0	0	0	0	9	
T. alba	2	7	0	6	2	2	2	1	2	0	1	0	1	39	

Podargus strigoides

P. strigoides chicks were taken from their enclosures individually, and rested on a towel, knitted hat, digital scale, log or directly on to a workbench (Figure 4-1). The touch experiment was filmed using GoPro Hero 5 camera (60 fps), and undertaken by zoo staff. There was some variation in this sequence and protocol, as the zoo staff changed each day and over the three years during which the experiment took place.

Tyto alba

All procedures were approved by the Science and Engineering Research Ethics and Governance Committee at Manchester Metropolitan University (EthOS reference 0445). To avoid stressing the chicks more than necessary, the chicks were not removed from their boxes during the experiment, which also contained them when they moved away from the cotton bud. The experiment was carried out a few days apart (2-5 days) and only once the parents had left the box during the day. For each chick, in most cases the experiment lasted less than a minute; however, on occasion i.e. in: cases where the chicks moved away or attacked (bit, flapped its wing, grabbed with its feet), the touch experiment for a chick could last no more than 2 min as the subsequent touch was done only once the chick calmed and settled back down (body standing still, the head could be bobbing), so that the chick did not hurt itself (fell from the box, walk over or squeezed by its siblings) or the experimenter. The mortality rate of the chicks was high (about 65%) in 2019 due to some environmental issues (e.g. parents being killed by rodenticide in field nearby, therefore they were not fed). Out of the 45 eggs laid, 40 hatched of which 24 chicks died; the 29 unhatched eggs and dead chicks corresponded to 3 different broods (one of 9, one of 8 , one of 7) and 5 deaths (3 eggs, 2 chicks) within a brood of 11. Out of the 16 surviving chicks, 10 fledged when I was present and 6 were about to fledge when I left.

T. alba chicks were filmed with a GoPro Hero 3 camera (60 fps), every 2 to 5 days. Since these chicks were wild, the experiment took place within their nest boxes *The Barn owl project* in Israel provides breeding boxes and these boxes were ideal for the touch experiment as they were accessible with a ladder and provided access to several chicks from different breeding pairs. Each chick of the clutch was stimulated by the cotton bud in turn within the box (Figure 4-1). Individuals could be recognised by their ages, so the same chicks were observed over time.

4.2.3 Characterisation of the development stages

The videos were studied at each age to develop a schedule of development for the feathers, rictal bristle emergence and eye-opening. Ordinal categories were developed for each of these measures and defined below. Both species had four clear periods based on the observations of four body feather phenotypes, which were defined here based on common chick development phases as hatchling, nestling, young juvenile and fledgling/immature (see Introduction Section 4.1). Hatchling chicks were covered with white down feathers in *P. strigoides* and bare skin with strips of white down feathers in *T. alba*. The nestling stage corresponded to the emergence of grey/brown feathers, i.e. juvenile feathers, superseding the white down feathers. In the young juvenile stage, the white down feathers disappeared completely superseded the juvenile feathers. In the last stage, fledgling/immature stage, all juvenile feathers have moulted, only adult feathers are covering the body.

Three stages of rictal bristle emergence were identified in both species, including absence, emergence and presence. Rictal bristle absence corresponded to the period where the region was bare. The emergence stage corresponded to the period where the small outgrowth of skin (papilla) emerged and from which the feather started growing. The rictal bristle presence stage corresponded to the period where the rictal bristles were fully developed, the feather papilla having been completely pushed away by the bristles.

The two species differed in the eye-opening stages. Four stages of eye-opening were categorised in *P. strigoides* and three were categorised in *T. alba*. In *P. strigoides*, the four stages of eye-opening consisted of a stage where the eyes were closed and three stages with their eyes opened (open 1-3). In the second stage, termed open 1, the eyes had recently opened and were still small and entirely black. In the third stage, open 2, the eyes are bigger and a coloured iris appeared. In the fourth stage, open 3, the eyes reached their adult size with a clear coloured iris present and eyelashes. In *T. alba*, the three stages consisted of a stage where the eyes were closed, and two stages with eyes opened (open 1 and 2). In the second stage, open 1, the eyes had recently opened and were still small and black. In the third stage, the eyes grew larger, a coloured iris appeared and the eyelashes emerged.

4.2.4 Adult bristle morphology and follicle anatomy

Adult rictal bristle morphology and follicle anatomy were also described in the two species. Rictal bristle morphology was described using four *P. strigoides* museum specimens from the World Museum of Liverpool and four *T. alba* specimens found dead (from e.g. road kill or poisoning) in the Hula Valley, Israel and stored frozen. The shape of the rictal bristles, i.e. branched, unbranched and barbs at the base, was documented (as per Chapter 3), and the rictal bristle length per species was measured with callipers for the three longest rictal bristles on each side of each specimen, then averaged, giving a total mean bristle length.

The description of the dissection, the histology procedure and acquisition of the microscope images has previously been documented in Chapter 3 for *P. strigoides*. The *T. alba* rictal bristle region was obtained from a dead specimen found within the ground of the Hula Valley, Israel and frozen for a number of years before dissection. The rictal region was dissected from a defrosted Israeli specimen and stored in PFA 4% (diluted with Phosphate Buffer Solution). The tissue was then mounted, sliced and stained with Masson's trichrome following the same procedure as Chapter 3. The same characterisation of the follicle anatomy was also carried out (Chapter 3) by identifying the components immediately surrounding the bristle follicle, including a description of the tissue type, musculature and mechanoreceptors.

4.2.5 Characterisation of the behavioural responses to touch

All the videos were reviewed and a single observer scored all the videos according to a single standardised ethogram that identified all the response behaviours of *P. strigoides* and *T. alba* chicks during their development (Figure 4-3; Table 4-2). The ethogram (Table 4-2) was developed specifically for this study and was based on five categories of behaviours, which were feeding, foraging, avoidance, annoyance and displacement. For each chick per day, the behavioural response to the cotton bud touch was counted as present or absent at each touch point position (Figure 4-2). Therefore, for each chick, the total number of occurrences of a behavioural response was recorded at each touch point position for that day. The number of touches, i.e. total number of times the cotton bud touched a given touch point position per chick and per video, was also recorded as this sometimes varied, especially in the *P. strigoides*.

Figure 4-3. Snapshot photograph *Podargus strigoides* illustrating three examples of behavioural responses to touch, A) mouth movement and turning beak towards, **B)** looking towards. The arrows represent the movements.

Table 4-2. Combined ethogram with a description of behavioural responses observed when touch with a cotton bud, over the development period of the *Podargus strigoides* and *Tyto alba* chicks.

Categories	Behaviour	Description (as observed in videos)
FEEDING	**Mouth movement**	The bird opens and closes its mouth in response to the stimulus in an attempt to catch cotton bud (Figure 4-3A)
	Bite	The bird succeeds in catching the cotton bud by closing its beak onto it.
	Swallow	The bird tries to ingest the cotton bud once caught, with a throat movement.
	Lift body up	The bird lifts its body upwards, erecting its head high without moving its feet, i.e. no displacement
FORAGING	**Look towards**	The bird moves its head up or down, looking towards the cotton bud (Figure 4-3B)
	Turn beak towards	The bird slightly tilts its head sideways, following the touch of the stimulus beak first (Figure 4-3A)
AVOIDANCE	**Shake head**	The bird moves its head side to side
	Retract head	The bird lowers its head down into its shoulders or slightly backwards, away from the stimulus
	Avoid	The bird leans its whole body backwards or sideways, away from the stimulus
	Wing movement	The bird moves its wing up and down in a stretch or a rapid movement, i.e. twitch
	Step away	The bird moves or shuffles away, making at least one step away from the stimulus
ANNOYANCE	**Move foot**	The bird moves its foot up and down in an attempt to grab, or stomping, sometimes kicking towards the cotton bud
	Beak clap	The bird makes a click sound with its beak by opening and closing it, sometimes its tongue can be out. No intention of catching the cotton bud is made.
	Vocal sound	The bird makes plaintive noises, crying for attention or makes a loud shriek
NO RESPONSE	**No response**	The bird does not show any sign of response

4.2.6 Statistical analysis

The effect of age (day 1-58), touch point position (top of the head, abdomen, rictal region and proximal region; Figure 4-2), rictal bristle emergence (absence, emergence, present) and eye-opening stages (closed, open but small, open but bigger to adult size) on the behavioural response were investigated. Age, touch point position, rictal bristle emergence and eye-opening stages were included as separate fixed effects in a linear mixed effects model, with the chick ID as random effect. These models were conducted using the *lmer* function (lme4 package; Bates *et al.*, 2015) in R Studio software (R Core Team, 2018). *Lmer* was selected as it accounts for inter-individual variability in behavioural responses by including random effects. Moreover, the data in this study was not normally distributed (checked using shapiro-wilk Test; (Shapiro and Wilk, 1965)) and had missing data points, but *lmer* is flexible and can accommodate both non parametric data and missing data points, so that chicks with data for at least one touch point can be included in the analysis. Of the 15 behaviours (Table 4-2), only behaviours displayed by both species, *P. strigoides* and *T. alba*, and occurring more than 30 times, were used in the models. The behaviours shake head, retract head, avoid, wing movement and step away (Table 4-2) were merged together as avoidance behaviours for the statistical analysis and corrected with the total number of times the region was touched (i.e. maximum of times the behaviour could have occurred). In total, the data of seven behavioural responses were included in the statistical analysis, including mouth movement (Figure 4-3), looking towards (Figure 4-3) the stimulus, turning beak towards the cotton bud (Figure 4-3), avoidance (including retract head, avoids, wing movement, and steps away), beak clapping and no response (Table 4-2). To visualise the data, the percentage of occurrences of each behavioural response (the sum of occurrences per behaviour was divided by the sum of number of hit) was calculated and then plotted against different fixed effects (touch point positions, development of feathers, rictal bristles emergence and eye-opening stages), using the *ggbarplot* function (ggpubr package; Wickham, 2016).

Ethogram reliability analysis

To assess the reliability of the ethogram, four videos for each species at the four different stages of chick development were chosen at random from the dataset. Six behavioural responses were tested (as per decision in 4.2.6), including mouth movement, look towards, turn beak towards, avoidance, beak clap and no response, for each of the touch point position. The videos were scored again by the same single observer to obtain the intra-reliability and scored by another observer to obtain the inter-observer reliability. Scores were then compared using Cronbach’s α (Table 4-3). As shown in the table, all reliability scores were greater than 0.8 indicating that all six measures had a high internal consistency as per Cronbach (1951). Subsequently, I am confident that the ethogram is robust enough to be scored by a single observer.

Table 4-3. Intra- and inter-observer reliability analysis scoring eight videos, four of *P. strigoides* (F) and four of *T. alba* (O) chicks during their development.

Behaviour	Species	Cronbach’s α scores	
		Intra-observer	Inter-observer
Mouth movement	F	0.95	0.93
	O	0.99	0.96
Look towards	F	0.99	0.92
	O	1	1
Turn beak towards	F	1	0.82
	O	0.94	0.97
Avoidance	F	0.97	0.98
	O	0.99	0.99
Beak clap	F	1	1
	O	1	1
No response	F	0.89	0.96
	O	0.99	0.99

4.3 Results

Results, here, will firstly describe and compare the developmental schedule of *P. strigoides* and *T. alba* chick by documenting the development of the body feathers, eyes, and rictal bristles. The presence of mechanoreceptors in both adult species will then be identified. Finally, the chicks’ behavioural responses to touch will be discussed in both species from birth to day 55-60.

4.3.1 Chick developmental schedule

The development of *P. strigoides* chicks consisted of four different stages, from hatchling to nestling, to young juvenile, to fledgling/immature, defined according to the four different development stage of body feathers observed (Figure 4-5A). There were also three clear stages of rictal bristle emergence (Figure 4-4, 5A) and four different stages of eye-opening (Figure 4-5A). The hatchling stage occurred from birth to day 7, during which time the hatchling had its body covered with white, down feathers and opened its eyes around day 6-7 (Figure 4-5A). At this stage, their eyes were dark and small with no coloured iris visible. The nestling stage was from day 7-8 to day 14, throughout which grey body feathers emerged, slowly replacing the white down feathers (Figure 4-5A). During this stage, the eyes grew and a coloured iris appeared around day 11 (Figure 4-5A). Next, the chick entered the young juvenile stage, attaining the final stage of eye-opening on day 15, which was adult-sized eyes with clear coloured irises and the appearance of eyelashes around the eyes (Figure 4-4). The young juvenile chick also displayed a body covered with grey and brown body feathers, with the total disappearance of white down feathers from day 15 to 31 (Figure 4-5A). The rictal bristles started to emerge from day 23 (Figure 4-4). During fledging, the chicks reached and exhibited a complete adult plumage - a cryptic grey and brown mixture of coloured feathers covering the whole body (Figure 4-5A). The fledgling rictal bristles finished their development around day 35.

T. alba chicks developed through the same development stages, although the schedule timing was slightly different, and there were only three stages of eye-opening (Figure 4-5B). During the hatchling stage, from birth to day 14, chicks displayed a bare, pink skin with scarce white down feathers (Figure 4-5B). During that stage, the hatchling opened its eyes around day 11, which were small without eyelashes (Figure 4-4D; Figure 4-5B). The pink skin was next fully covered with white down feathers at the nestling stage from day 15 to 19 (Figure 4-5B). The chick moved to the young juvenile stage from day 20 to day 45, where grey/brown flight feathers developed on the wing. At this stage, rictal bristles emerged and developed from day 26 to 35, and the eyes continued to grow, reaching an adult size eye at day 32 with the appearance of eyelashes (Figure 4-4D-F; Figure 4-5B). In the fledgling stage, the chick displayed a complete adult plumage with the total disappearance of the down feathers from day 46 (Figure 4-5B). The adult plumage displayed a white facial disk, wing underside and abdomen, which can be spotted in some individual, whereas the facial ruff and the whole back of the bird from the back of the head, back of the wing and tail were beige/light brown with grey spots (Figure 4-5B).

Compared to *T. alba*, *P. strigoides* chicks developed faster (Figure 4-5). However, the emergence of the rictal bristles was faster in *T. alba* (9 days) than in *P. strigoides* (12 days), although rictal bristles started to emerge three days sooner in *P. strigoides* (around day 23) than in *T. alba* (Figure 4-4B, E; Figure 4-5). Indeed, rictal bristles were still developing once *P. strigoides* had fledged the nest while *T. alba* had its rictal bristles fully developed during the young juvenile stage and before fledging (Figure 4-4C, F; Figure 4-5).

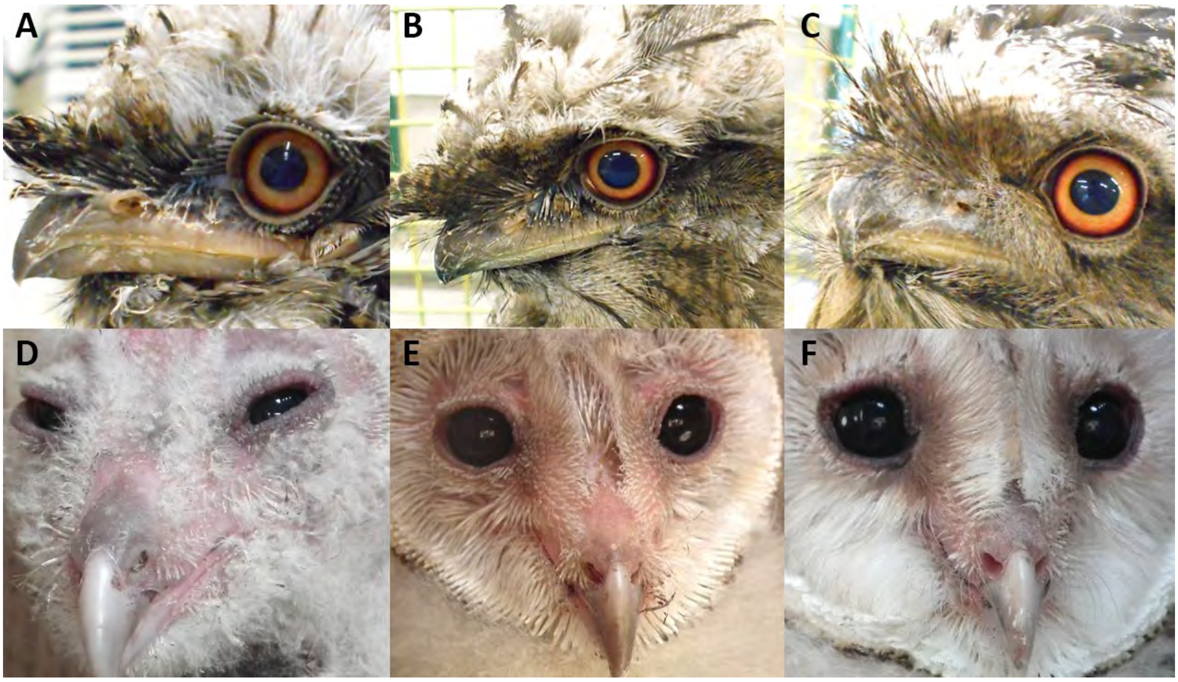


Figure 4-4. Rictal bristle emergence illustrated by photographs of A-C) *Podargus strigoides* and D-F) *Tyto alba* chicks. Their emergence comprises the stages: **A), D)** Absence, **B), E)** Emergence, **C)** and **F)** Presence.

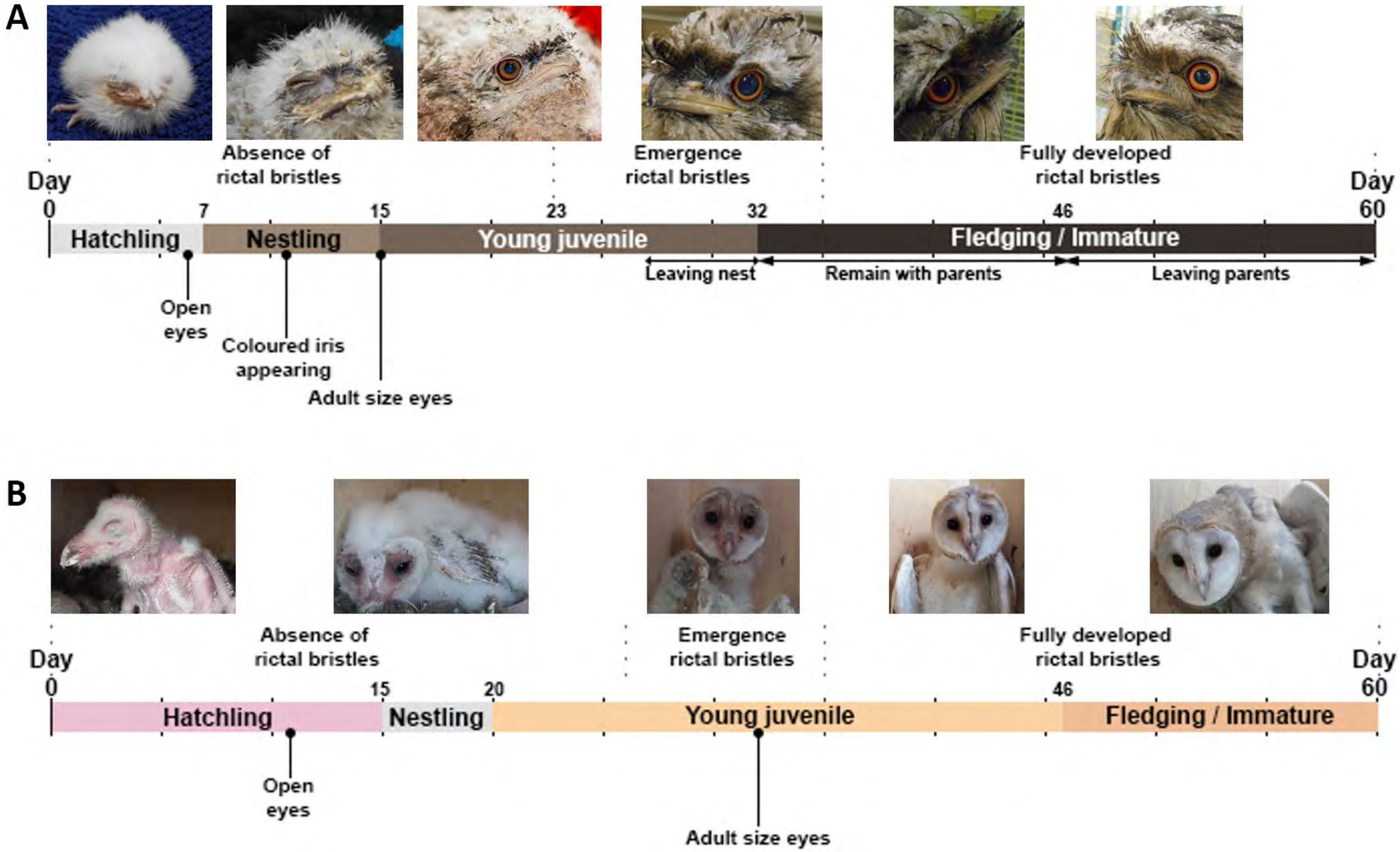


Figure 4-5. Timelines graphs representing the different stages of the development of the A) *Podargus strigoides* and B) *Tyto alba* chicks, illustrating the evolution of chick plumages from hatchling to nestling to young juvenile to fledgling/immature. The rictal bristle emergence and eye-opening are also illustrated on the timeline.

4.3.2 Adult rictal bristle morphology and anatomy

As stated in Chapter 3, the rictal bristle-bearing integument of *P. strigoides* revealed a dense tissue, i.e. low quantity of adipose tissue (Table 4-4; Figure 4-6A-B) with the smooth erector or depressor muscles running diagonally from consecutive rictal bristle follicles and the smooth apterial bristle muscle joining the distal tips of the rictal bristles (Table 4-4; Figure 4-6A-B). *P. strigoides* dermis also contained connective tissue around the follicles (blue ring around the follicles; Table 4-4; Figure 4-6A-B) and about four mechanoreceptors, i.e. Herbst corpuscles, in its immediate surroundings (Table 4-4; Figure 4-6A-B). *P. strigoides* had dark brown, branched rictal bristles (Figure 4-6C). The mean rictal bristle length measured for the species was 23.2mm ± 1.2 SD.

In comparison, *T. alba* rictal bristle-bearing integument consisted of a more porous dermis with a high concentration of adipose tissue (Table 4-4; Figure 4-7A-B). The bundle of smooth erector or depressor muscle fibres appeared to be of a similar size to *P. strigoides* and connected the bristle follicles together (Table 4-4; Figure 4-7A-B). There was no apparent layer of connective tissue at the follicle (Table 4-4; Figure 4-7A-B). *T. alba* had seven Herbst corpuscles, present all around the rictal bristle follicles (Table 4-4; Figure 4-7A-B), which was more than *P. strigoides*. *T. alba* had white, unbranched rictal bristles (Figure 4-7C). The mean rictal bristle length measured for the species was 17.4mm ± 7.0 SD.

Table 4-4. Summary table of the anatomical descriptions of the bristle-bearing integument of the two species, *Podargus strigoides* and *Tyto alba*. HC is Herbst corpuscles. HC position corresponds to the position of Herbst corpuscles at the bristle follicle. HC number corresponds to the total of Herbst corpuscles count for one follicle.

Species / Components	*P. strigoides*	*T. alba*
Adjacent muscle	Smooth erector/depressor muscle Smooth apterial muscle	Smooth erector/depressor muscle
Fibres bundle	Large	Large
Connective tissue	Present	Absent
HC position	Around	Around
HC number	4	7
Dermis density	Dense	Porous
Quantity of adipose tissue	20%	60%

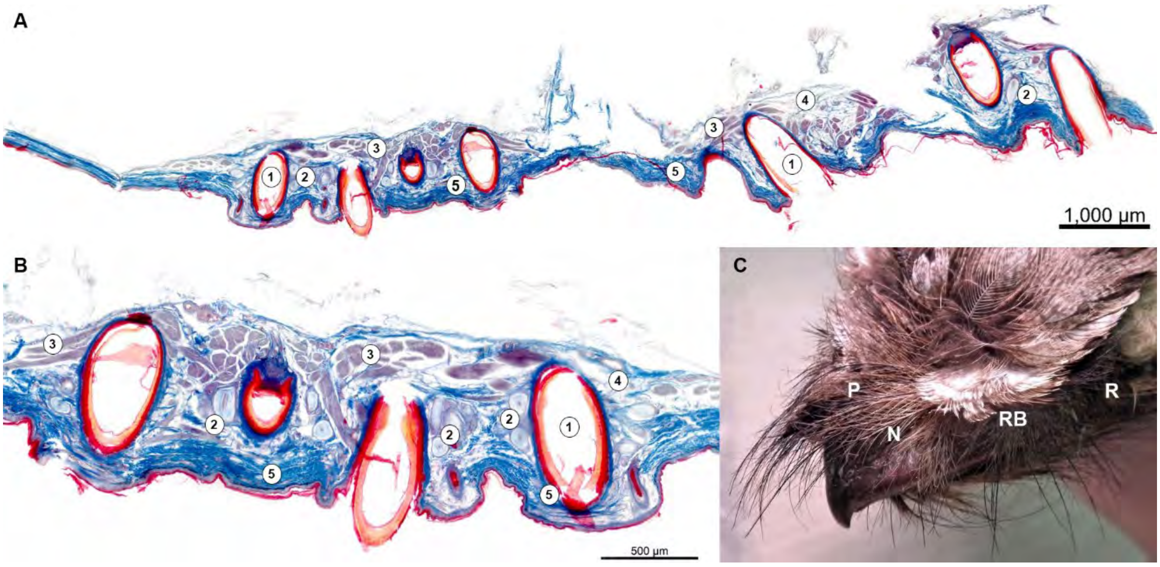


Figure 4-6. Follicle anatomy and morphology of *Podargus strigoides* rictal bristles (**RB**). **A)** Longitudinal section of *P. strigoides* rictal bristle follicles from nares (**N**) to rictus (**R**). **B)** Enlargement of the row of rictal bristle follicles (**1**), which reveals the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), dermal muscle fibres (**3**), adipose tissue (**4**) and connective tissue (**5**) surrounding the follicles. Sections stained with Masson's trichrome stain. **C)** Photograph of an adult museum specimen illustrating the branched morphology of the rictal bristles (**RB**), the proximal bristles (**P**, i.e. bristles between the eyes, extending forward above and in the alignment of the beak tip), and the narial bristles (**N**, directly above the nares, covering the orifice).

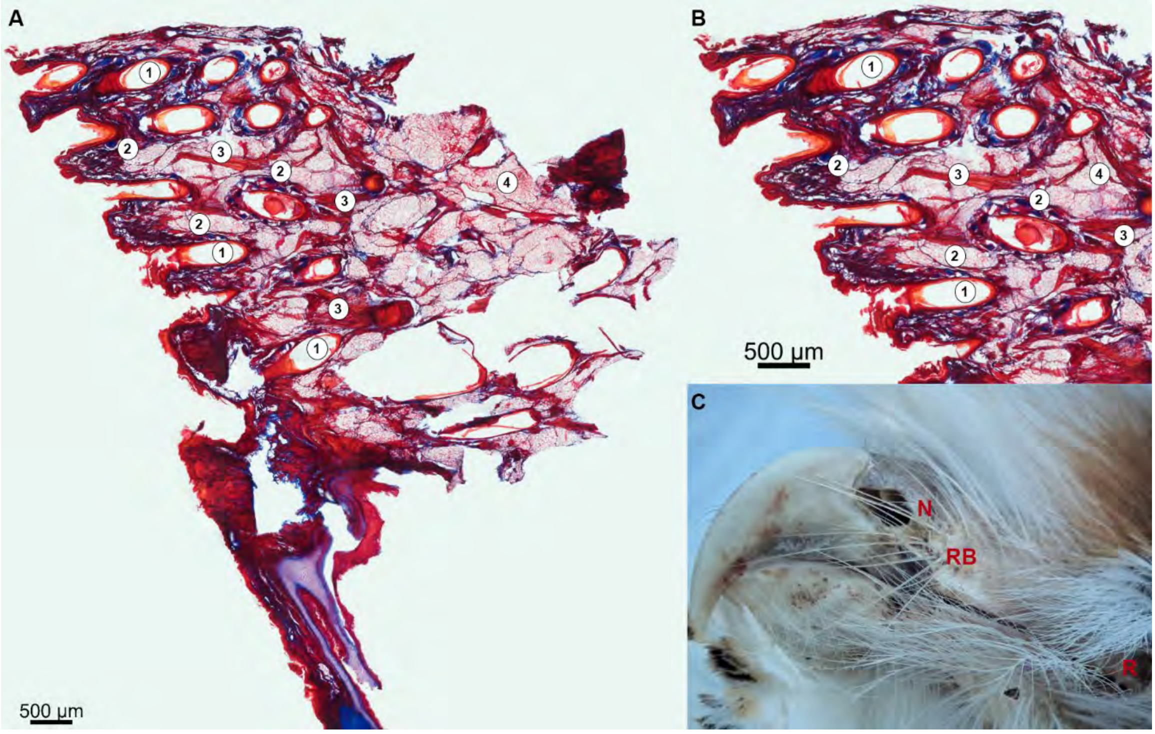


Figure 4-7. Follicle anatomy and morphology of the *Tyto alba* rictal bristles (**RB**). **A)** Longitudinal section of *T. alba* rictal bristle follicles from nares (**N**) to rictus (**R**). **B)** Enlargement of the row of rictal bristle follicles (**1**), revealing the presence of mechanoreceptors, i.e. Herbst corpuscles (**2**), dermal muscle fibres (**3**) and adipose tissue (**4**) in the follicles surroundings. Sections stained with Masson's trichrome stain. **C)** Photograph of an adult specimen illustrating the unbranched morphology of the rictal bristles (**RB**) and the narial bristles (**N**, directly above the nares, covering the orifice).

4.3.3 Chick behavioural responses to touch

Touch point position

P. strigoides chicks exhibited a more diverse behavioural response when they were touched on the rictal region, top of the head and proximal region compared to the abdomen (Figure 4-8A). *P. strigoides* chicks did not respond to touch on the abdomen 89.5% of the time (Figure 4-8A). *T. alba* chicks displayed less diverse behavioural responses when touched on the abdomen (with no response 83.9% of the time), top of the head (62.8% no response), and proximal region (72% no response) compared to the rictal region (Figure 4-8B). In these regions, *T. alba* chicks reacted by avoiding the cotton bud touch (7.85%, 37.2%, 25.9%, respectively), if reacting at all.

In both species, mouth movements and turning the beak towards the cotton bud significantly increased when touched on the rictal region compared to any other region (*p*<0.001; Table 4-5A; Figure 4-8). Looking towards the cotton bud increased when touched on the abdomen (*p*<0.001) and the proximal region (*p*=0.036) in *T. alba*, and touched on the top of the head (*p*<0.001) and the proximal region (*p*<0.001) in *P. strigoides* (Table 4-5A; Figure 4-8). Avoidance significantly occurred more when the chicks were touched on the rictal region compared to the abdomen in both species (*p*≤0.001), and on the proximal region in *P. strigoides* (*p*<0.001); whereas *T. alba*, avoided the cotton bud significantly less when touched on the rictal region compared to the top of the head and the proximal region (*p*≤0.001; Table 4-5A; Figure 4-8). *P.* strigoides significantly clapped their beak more when touched on the rictal region than on any other region (*p*<0.001; Table 4-5A; Figure 4-8). Finally, both species were significantly more unresponsive when touched on the abdomen and top of the head than on the rictal region (*p*<0.001; Table 4-5A; Figure 4-8); *T. alba* were additionally significantly more unresponsive when touched on the proximal region than the rictal region (*p*<0.001; Table 4-5A; Figure 4-8B).

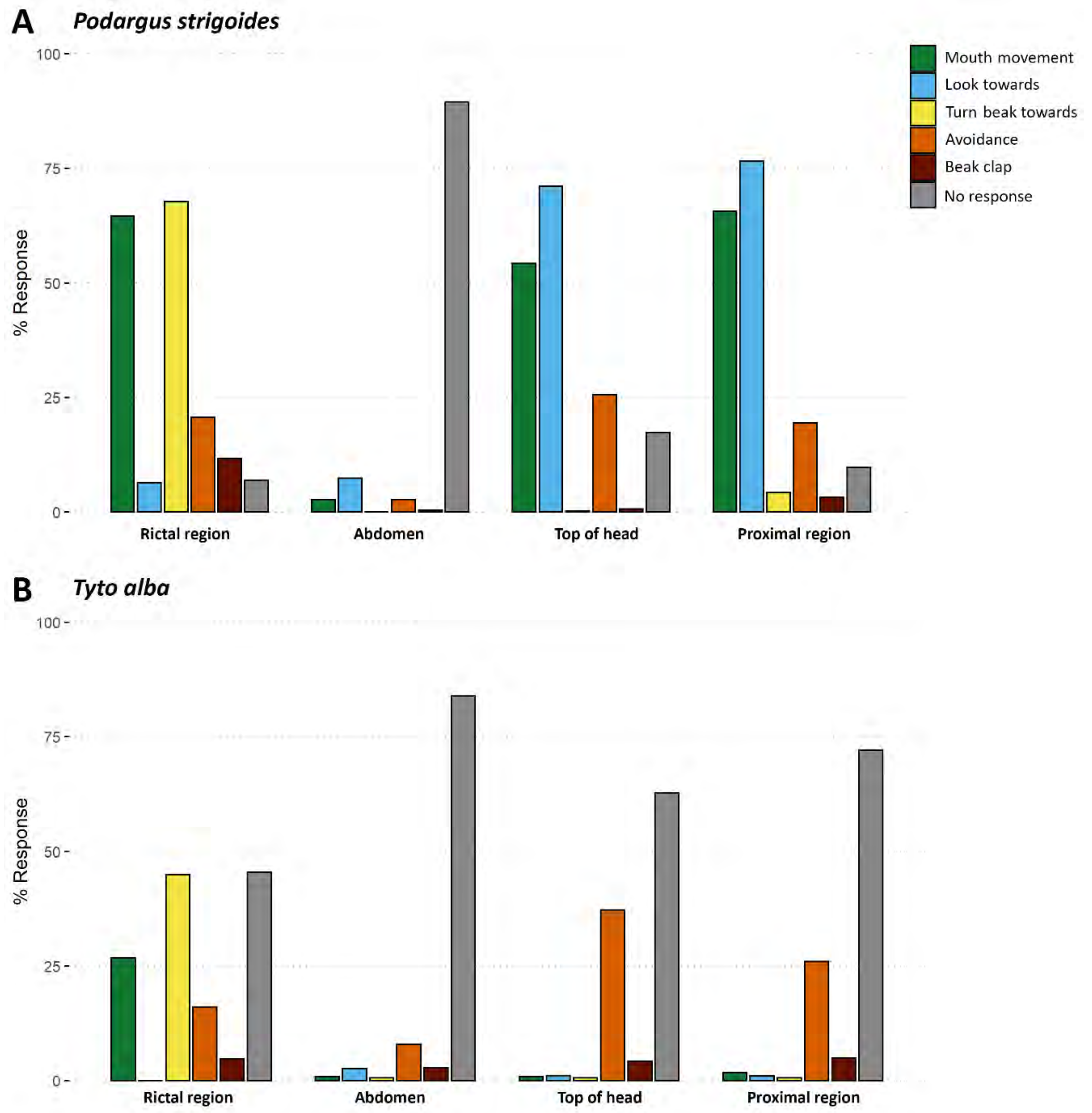


Figure 4-8. Response behaviour (%) **to touch of A)** ***Podargus strigoides*** **and B)** ***Tyto alba*** **chicks when touched on four different regions**: rictal region, abdomen, top of the head, and the proximal region. The response behaviours included the behaviours: mouth movement, look towards, turn beak towards, avoidance, beak clap, and no response.

Table 4-5. **Summary of the effects of age (model B) and touch point positions (model A) on the *Podargus strigoides*** (F) **and *Tyto alba*** (O) **chicks' behavioural responses, in reference to touch on the rictal region.** Behavioural responses include mouth movement, look towards, turn beak towards, avoidance, beak clap and no response. *P*-values and estimates are from a lmer model with N=9 for the frogmouth and N=41 for the barn owl; *p-values* in bold indicate the significant ones.

	Behaviour		**Mouth movement**		**Look towards**		**Turn beak towards**		**Avoidance**		**Beak clap**		**No response**	
	Tests		Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value
A	**Abdomen**	F	-59.27	**<0.001**	1.07	0.726	-64.88	**<0.001**	-15.00	**<0.001**	-9.12	**<0.001**	57.41	**<0.001**
		O	-24.12	**<0.001**	2.49	**<0.001**	-42.31	**<0.001**	-8.20	**0.001**	-2.12	0.053	36.46	**<0.001**
	Head	F	-24.12	**<0.001**	43.94	**<0.001**	-64.68	**<0.001**	1.33	0.578	-8.98	**<0.001**	7.01	**0.009**
		O	-24.17	**<0.001**	1.04	0.103	-42.17	**<0.001**	19.71	**<0.001**	-0.54	0.621	16.81	**<0.001**
	Proximal	F	-21.19	**<0.001**	42.21	**<0.001**	-62.44	**<0.001**	-5.11	**0.032**	-7.86	**<0.001**	1.28	0.632
		O	-23.49	**<0.001**	1.33	**0.036**	-42.18	**<0.001**	8.40	**0.001**	-0.19	0.864	23.96	**<0.001**
B	**Age**	F	-0.20	0.053	-0.46	**<0.001**	0.08	0.297	0.04	0.543	0.12	**<0.001**	0.05	0.592
		O	0.40	**<0.001**	0.01	0.518	0.46	**<0.001**	0.72	**<0.001**	0.07	**0.033**	-0.90	**<0.001**

Age

As the chicks got older, and went through different stages of development (1.hatchling, 2.nestling, 3.young juvenile, 4.immature; Figure 4-9), both species significantly beak clapped more when touched (*p*<0.001 in *P. strigoides*, *p*=0.033 in *T. alba*; Table 4-5B; Figure 4-9). *T. alba* also significantly increased their mouth movements (*p*<0.001), turning beak towards (*p*<0.001), and avoidance (*p*<0.001), and decreased their unresponsiveness (*p*<0.001) as they got older (*p*<0.001; Table 4-5B; Figure 4-9D-F). However, *P. strigoides*, looked towards the stimulus less as they got older (*p*<0.001; Table 4-5B; Figure 4-9A-C).

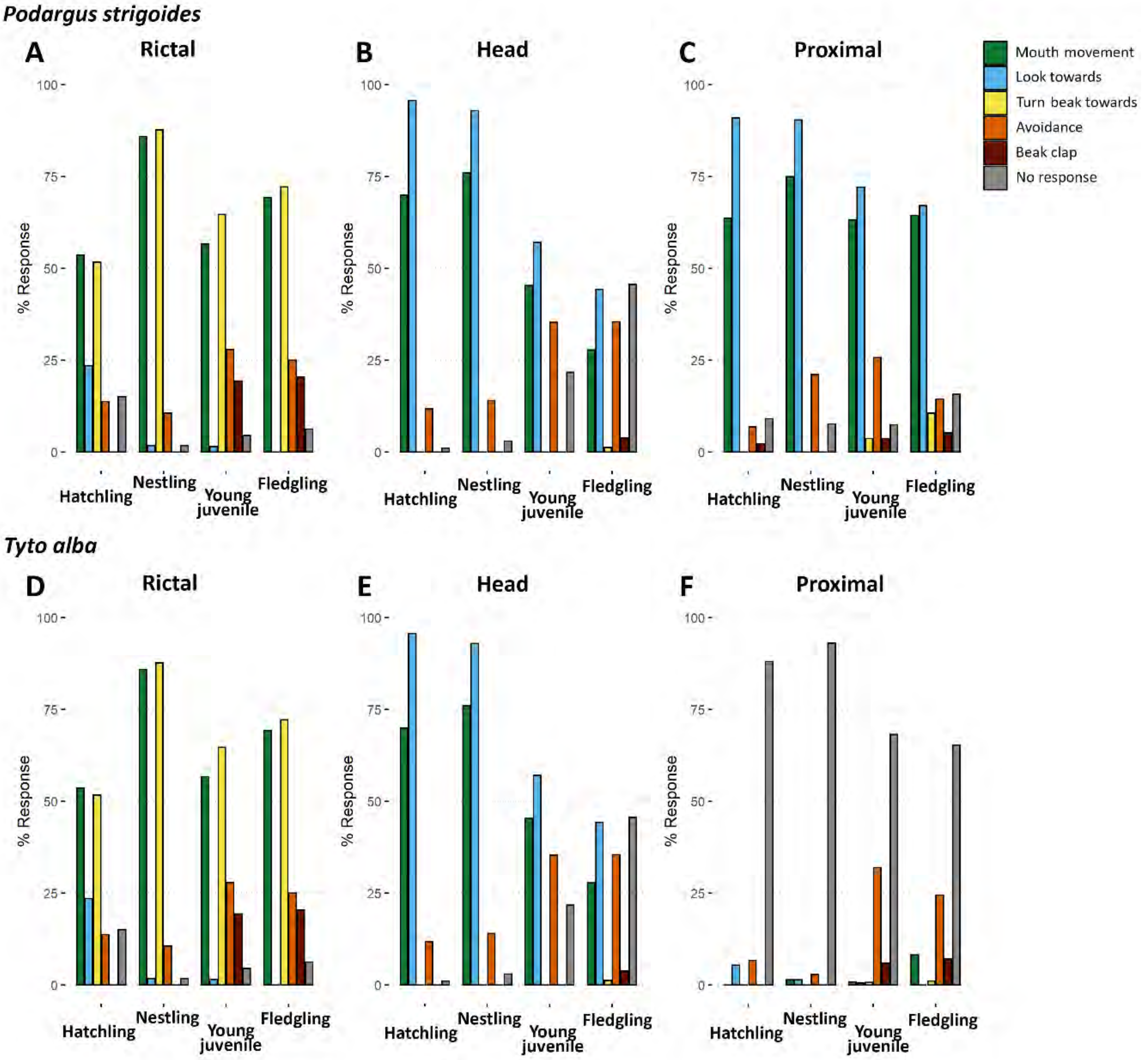


Figure 4-9. **Behavioural responses** (%) **of *Podargus strigoides* (A-C) and *Tyto alba* (D-F) chicks to touch at different stages of development of feathers.** Region touched with the cotton bud included: **A,D)** the rictal region, **B,E)** the top of the head, **C,F)** the proximal region. The behavioural responses were mouth movement, look towards, turn beak towards, avoidance, beak clap, and no response.

Rictal bristle emergence

As the rictal bristles emerged and became fully grown, *P. strigoides* increased beak clapping (*p*<0.001; Table 4-6; Figure 4-10), especially when touched on the rictal region compared to any other region (*p*<0.001; Table 4-6; Figure 4-10). *T. alba* chicks increased their behavioural responses, specifically their mouth movements (*p*<0.001), turning their beak towards (*p*<0.001), avoidance (*p*=0.010), and decreased their unresponsiveness when the rictal bristles emerged to fully grown (*p*<0.001; Table 4-6; Figure 4-10). *T. alba* made more mouth movements and turned their beak towards the cotton bud more when touched on the rictal region than any other region as the rictal bristles started to emerge (*p*<0.001; Table 4-6; Figure 4-10).

As the rictal bristles emerged, *P. strigoides* chicks also did fewer mouth movements (*p*<0.001) and look towards the cotton bud less (*p*<0.001) when touched on the top of the head and were less unresponsive when touched on the abdomen (*p*<0.001) than on the rictal region as the rictal bristles emerged (Table 4-6; Figure 4-10). However, *P. strigoides* were more unresponsive when touched on the top of the head than on the rictal region as the rictal bristles emerged (*p*<0.003; Table 4-6; Figure 4-10). When touched on the abdomen and the proximal region rather than the rictal region, *T. alba* were more unresponsive, as the rictal bristles emerged (*p*<0.001; Table 4-6). As the rictal bristles grew, touches on the top of the head and proximal region, rather than rictal region, were avoided more by *T. alba* (*p*=0.003), and they looked less towards the cotton bud when touched on the proximal region (*p*=0.016; Table 4-6; Figure 4-10).

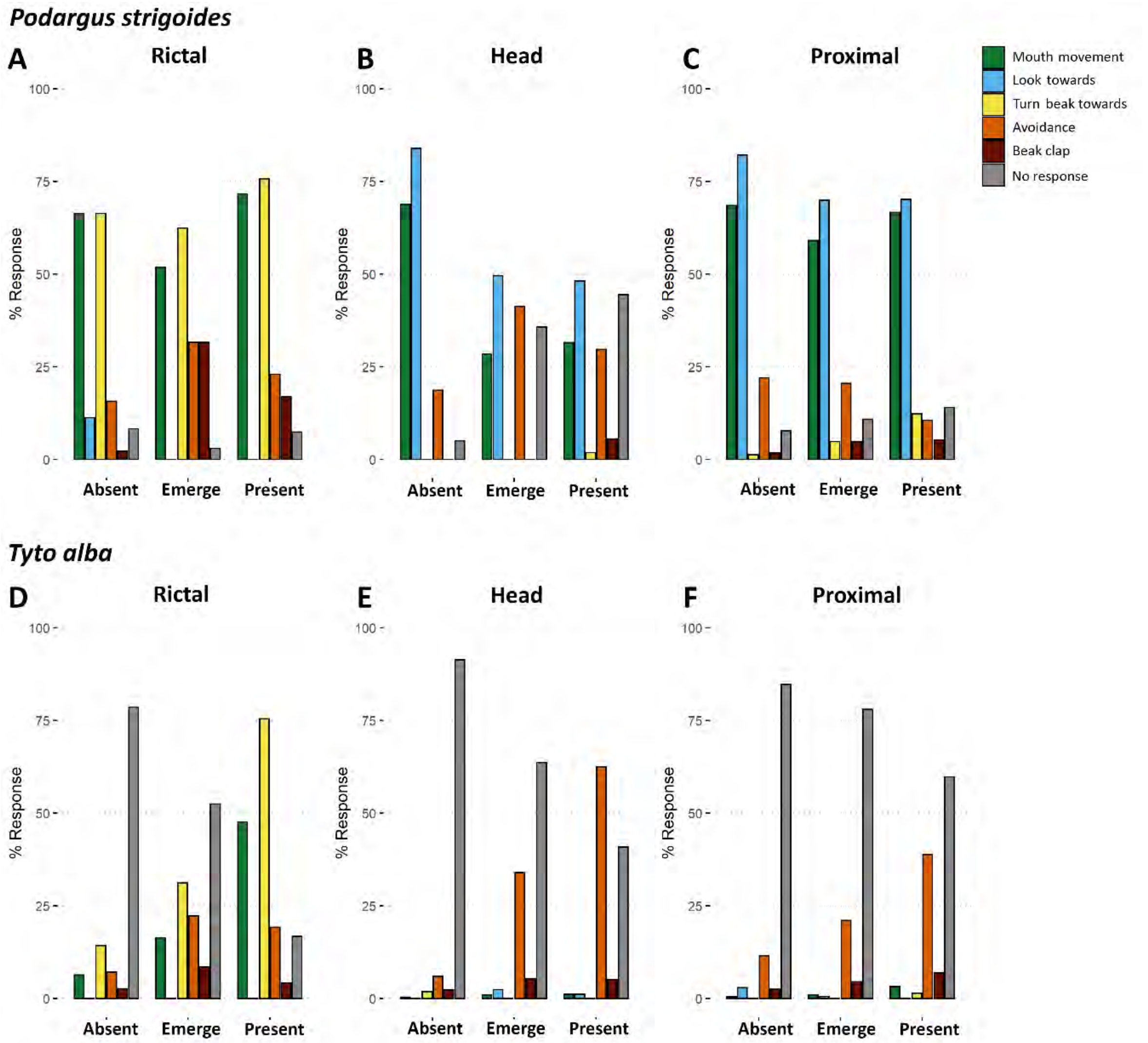


Figure 4-10. Behavioural response (%) **of *Podargus strigoides*** (**A-C**) **and *Tyto alba*** (**D-F**) **chicks to touch at the different stages of the rictal bristle emergence:** absent, emerge, present**.** Region touched included: **A,D)** rictal region, **B,E)** top of the head, **C,F)** proximal region. Response behaviours were mouth movement, look towards, turn beak towards, avoidance, beak clap, and no response.

Table 4-6. **Summary of the effects of rictal bristle** (RB) **emergence and touch point positions on *Podargus strigoides*** (F) **and *Tyto. alba*** (O) **chicks' behavioural responses, in reference to touch on the rictal region.** Behavioural responses include mouth movement, look towards, turn beak towards, avoidance, beak clap and no response. *P*-values and estimates are from a lmer model with N=9 for the frogmouth and N=41 for the barn owl; *p*-values in bold indicate the significant ones.

Behaviour **Tests**		**Mouth movement**		**Look towards**		**Turn beak towards**		**Avoidance**		**Beak clap**		**No response**	
		Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value
RB emergence	F	1.63	0.571	-2.29	0.387	2.14	0.223	0.30	0.888	6.49	**<0.001**	0.26	0.909
	O	19.32	**<0.001**	0.00	1.000	29.10	**<0.001**	5.09	**0.010**	0.49	0.592	-26.98	**<0.001**
Abdomen	F	-59.46	**<0.001**	-0.21	0.957	-63.62	**<0.001**	-14.90	**<0.001**	-4.91	**0.001**	65.19	**<0.001**
	O	-4.78	**0.037**	1.17	0.242	-11.47	**<0.001**	-2.59	0.486	-2.17	0.213	9.91	**0.016**
Head	F	-10.92	**0.009**	55.01	**<0.001**	-63.69	**<0.001**	1.84	0.551	-5.15	**<0.001**	0.69	0.839
	O	-4.03	0.078	0.49	0.628	-9.84	**<0.001**	-3.91	0.292	-0.89	0.609	12.28	**0.003**
Proximal	F	-19.20	**<0.001**	43.53	**<0.001**	-63.01	**<0.001**	-2.61	0.396	-4.05	**0.006**	-0.35	0.919
	O	-4.30	0.060	3.20	**0.001**	-12.07	**<0.001**	-0.27	0.942	-1.70	0.328	2.66	0.516
RB dev * Abdomen	F	0.28	0.945	1.92	0.601	-1.88	0.443	-0.15	0.960	-6.33	**<0.001**	-11.69	**<0.001**
	O	-18.20	**<0.001**	1.24	0.091	-29.02	**<0.001**	-5.29	0.051	0.04	0.974	25.00	**<0.001**
RB dev * Head	F	-19.84	**<0.001**	-16.65	**<0.001**	-1.48	0.546	-0.77	0.793	-5.76	**<0.001**	9.51	**0.003**
	O	-18.94	**<0.001**	0.52	0.477	-30.42	**<0.001**	22.24	**<0.001**	0.33	0.796	4.26	0.155
RB dev * Proximal	F	-2.99	0.456	-2.01	0.585	0.87	0.723	-3.77	0.200	-5.72	**<0.001**	2.46	0.446
	O	-18.06	**<0.001**	-1.76	**0.016**	-28.34	**<0.001**	8.15	**0.003**	1.42	0.262	20.05	**<0.001**

Eye-opening stages

In *P. strigoides*, the eye-opening stages affected the behavioural response. Once *P. strigoides* chicks opened their eyes, the chicks significantly decreased the occurrences of look towards ($p \leq 0.006$ for open 1-3) and unresponsiveness ($p \leq 0.002$, Table 4-7; Figure 4-11A-C), although the latter was not significant for the last stage of eye-opening. *P. strigoides* looked towards the cotton bud significantly more when touched on any other region than the rictal region once their eyes opened (stage 2-3). *P. strigoides*, however, increased turning their beak towards ($p \leq 0.001$) once their eyes were opened, especially when touched on the rictal region compared to any other region (at any given stage open eye stages $p=0.024$, Table 4-7; Figure 4-11A-C). *P. strigoides* also increased their mouth movements when their eyes were opened and before attaining the adult eye size (open 1, $p<0.001$ and open 2, $p=0.002$); once their eyes reached their adult size, *P. strigoides* beak clapped more ($p<0.001$, Table 4-7; Figure 4-11A-C) than when *P. strigoides* had their eyes closed. *P. strigoides* made significantly more mouth movements and were less unresponsive when touched on the rictal region than on the abdomen when their eyes were bigger but not yet at adult size (open 2, $p=0.021$ and $p<0.001$, respectively; Table 4-7), and did more mouth movements and were less unresponsive when touched on the rictal region than on top of the head when their eyes were at adult size (open 3, $p=0.021$ and $p<0.001$, respectively; Table 4-7). *P. strigoides* also clapped their beak more when touched on the rictal region than on any other region when their eyes attained adult size (open 3, $p<0.001$, Table 4-7). Finally, *P. strigoides* avoided significantly more when touched on the top of the head and on the proximal region than on the rictal region when their eyes were bigger, but not yet adult-sized ($p=0.031$ and $p=0.019$, respectively; Table 4-7).

In *T. alba*, the eye-opening stages had less of an impact on the behavioural responses. Indeed, only when the eyes had attained their adult size did *T. alba* make significantly more mouth movements ($p<0.001$), turning beak towards ($p<0.001$), avoidance ($p=0.014$), and were less unresponsive ($p<0.001$, Table 4-8; Figure 4-11D-F). *T. alba* significantly increased the occurrences of mouth movements and turned their beak more when touch on the rictal region than on any other region once their eyes were at adult size (open 2, $p<0.001$, Table 4-8). *T. alba* also avoided more when touched on the rictal region than on the abdomen once their eyes were opened ($p \leq 0.048$) and more when touched on the top of the head than on the rictal region when their eyes were at adult size (open 2, $p<0.001$, Table 4-8).

Finally, *T. alba* increased their unresponsiveness when touched on the abdomen and the proximal region once their eyes were at adult size (open 2, $p<0.001$, Table 4-8).

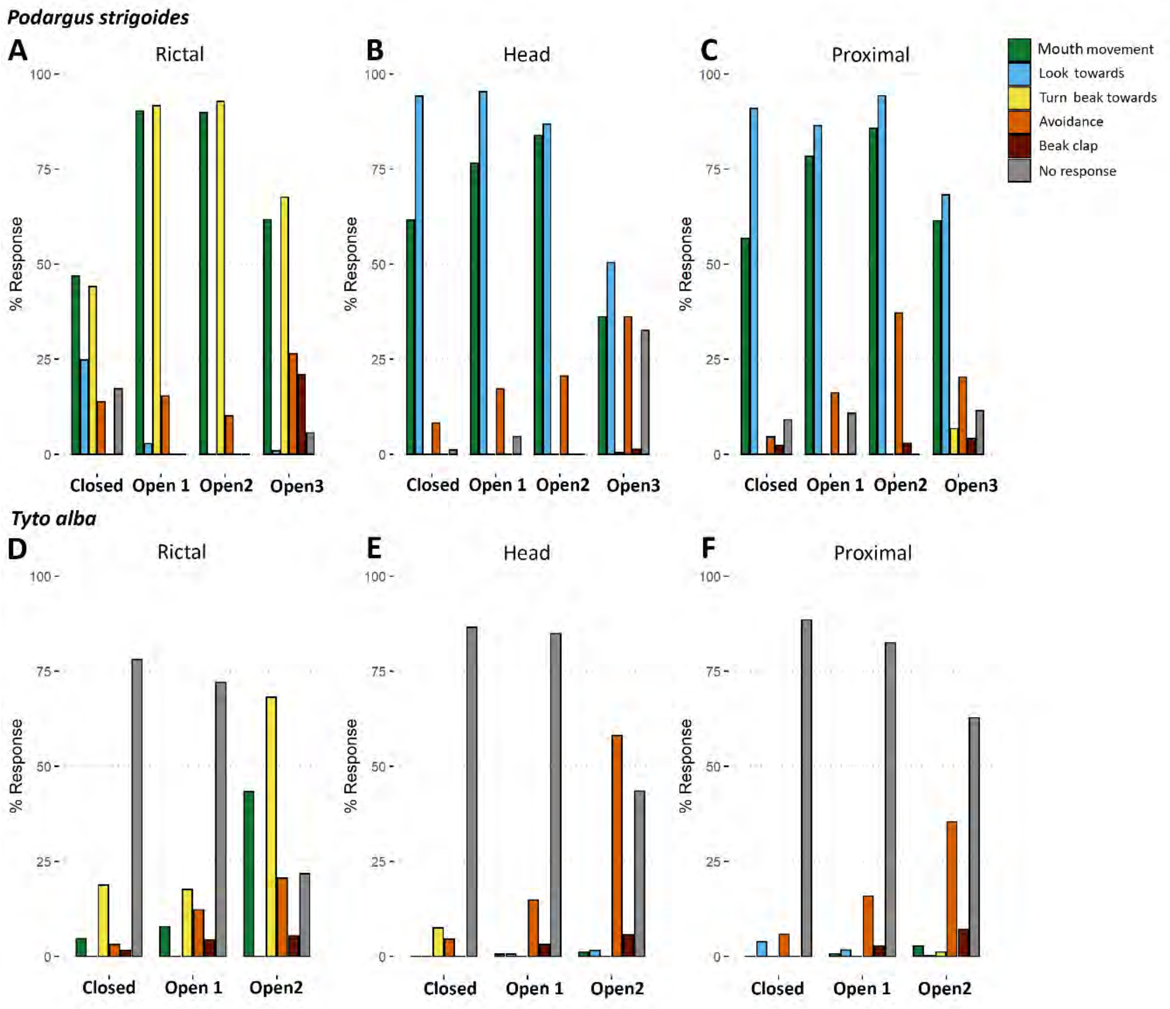


Figure 4-11. Behavioural responses (%) **of *Podargus strigoides*** **(A-C) and *Tyto alba*** **(D-F) chicks to touch at different stages of eye-opening.** Region touched with the cotton bud included: **A,D)** the rictal region, **B,E)** the top of the head, **C,F)** the proximal region. The eye-opening stages comprised closed, open 1 (hatchling), open 2 (nestling), open 3 (young juvenile) in *P. strigoides* and closed, open 1 (hatchling) and open 2 (young juvenile) in *T. alba*. The behavioural responses were mouth movement, look towards, turn beak towards, avoidance, beak clap, and no response.

4.3.4 Additional behavioural observations

Overall, *P. strigoides* chicks exhibited more diverse behaviours than *T. alba*. Specifically, they displayed swallowing, shaking head and vocal sound behaviours, that were not present in *T. alba,* whereas move foot only occurred in *T. alba* (Figure 4-12). Lift body up was performed by both species although it mainly occurred in *P. strigoides* (Figure 4-12).

Table 4-7. **Summary of the effects of eye-opening and touch point positions on *Podargus strigoides* chicks' behavioural responses, in reference to closed eyes and touch on the rictal region**. Behavioural responses include mouth movement, look towards, turn beak towards, avoidance, beak clap and no response. *P*-values and estimates are from a lmer model with N=9 for the frogmouth and N=41 for the barn owl; *p*-values in bold indicate the significant ones.

Behaviour	**Mouth movement**		**Look towards**		**Turn beak towards**		**Avoidance**		**Beak clap**		**No response**	
Tests	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value
Eyes open 1	31.09	**<0.001**	-22.21	**0.006**	38.74	**<0.001**	0.18	0.978	0.00	1.000	-16.18	**0.023**
Eyes open 2	27.12	**0.002**	-24.10	**0.003**	37.42	**<0.001**	-2.11	0.742	0.00	1.000	-15.44	**0.028**
Eyes open 3	10.54	0.105	-18.51	**0.002**	16.13	**<0.001**	7.34	0.122	15.64	**<0.001**	-9.51	0.066
Abdomen	-48.24	**<0.001**	-20.62	**0.004**	-46.21	**<0.001**	-12.31	**0.033**	0.60	0.828	42.24	**<0.001**
Head	-7.79	0.320	39.90	**<0.001**	-46.21	**<0.001**	-7.95	0.169	0.00	1.000	-14.93	**0.019**
Proximal	-18.88	**0.016**	21.45	**0.003**	-46.21	**<0.001**	-10.71	0.064	0.79	0.774	-7.79	0.222
Open 1 * Abdomen	-23.90	0.051	41.31	**<0.001**	-38.85	**<0.001**	-1.21	0.894	-0.60	0.890	17.83	0.074
Open 2 * Abdomen	-27.86	**0.021**	32.29	**0.004**	-37.65	**<0.001**	3.41	0.703	-0.60	0.889	41.10	**<0.001**
Open 3 * Abdomen	-8.28	0.350	21.85	**0.007**	-16.19	**0.002**	-4.94	0.450	-16.02	**<0.001**	13.73	0.057
Open 1 * Head	-12.63	0.302	33.96	**0.003**	-38.85	**<0.001**	7.33	0.418	-0.00	1.000	18.38	0.066
Open 2 * Head	7.79	0.521	41.50	**<0.001**	-37.65	**<0.001**	19.29	**0.031**	-0.00	1.000	14.93	0.131
Open 3 * Head	-26.45	**0.003**	-8.12	0.319	-15.85	**0.003**	10.25	0.117	-15.03	**<0.001**	30.18	**<0.001**
Open 1 * Proximal	-19.39	0.114	25.96	**0.021**	-38.85	**<0.001**	8.09	0.371	-0.79	0.855	12.37	0.215
Open 2 * Proximal	-9.99	0.410	38.55	**0.001**	-37.65	**<0.001**	20.98	**0.019**	0.31	0.941	7.79	0.431
Open 3 * Proximal	1.88	0.832	22.01	**0.007**	-12.11	**0.024**	3.62	0.580	-14.38	**<0.001**	11.22	0.120

Table 4-8. **Summary of the effects of eye-opening and touch point position on *Tyto alba* chicks' behavioural responses, in reference to closed eyes and touch on the rictal region.** Behavioural responses include mouth movement, look towards, turn beak towards, avoidance, beak clap and no response. *p*-values and estimates are from a lmer model with N=9 for the frogmouth and N=41 for the barn owl; *p*-values in bold indicate the significant ones.

Behaviour / **Tests**	**Mouth movement**		**Look towards**		**Turn beak towards**		**Avoidance**		**Beak clap**		**No response**	
	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value	Estimates	*p*-value
Eyes open 1	2.99	0.429	0.00	1.000	-4.73	0.268	8.31	0.183	1.94	0.503	1.57	0.820
Eyes open 2	35.85	**<0.001**	0.00	1.000	42.86	**<0.001**	15.13	**0.014**	2.54	0.373	-42.75	**<0.001**
Abdomen	-5.43	0.258	0.00	1.000	-22.04	**<0.001**	9.43	0.232	-2.17	0.552	9.04	0.296
Head	-5.43	0.258	0.00	1.000	-13.35	**0.014**	0.74	0.925	-2.17	0.552	11.96	0.167
Proximal	-5.43	0.258	3.61	0.088	-22.04	**<0.001**	1.83	0.817	-2.17	0.552	0.00	1.000
Open 1 * Abdomen	-3.12	0.557	1.72	0.464	4.56	0.446	-17.26	**0.048**	-0.27	0.947	9.33	0.330
Open 2 * Abdomen	-34.21	**<0.001**	3.53	0.124	-43.34	**<0.001**	-21.06	**0.014**	0.31	0.937	46.49	**<0.001**
Open 1 * Head	-2.48	0.642	0.65	0.782	-4.78	0.425	0.81	0.926	1.03	0.800	2.18	0.820
Open 2 * Head	-34.80	**<0.001**	1.53	0.505	-52.79	**<0.001**	36.59	**<0.001**	2.40	0.545	7.82	0.405
Open 1 * Proximal	-2.48	0.642	-1.33	0.569	3.92	0.513	0.79	0.928	0.29	0.943	9.15	0.340
Open 2 * Proximal	-33.48	**<0.001**	-3.42	0.137	-42.58	**<0.001**	12.26	0.152	3.67	0.355	39.85	**<0.001**

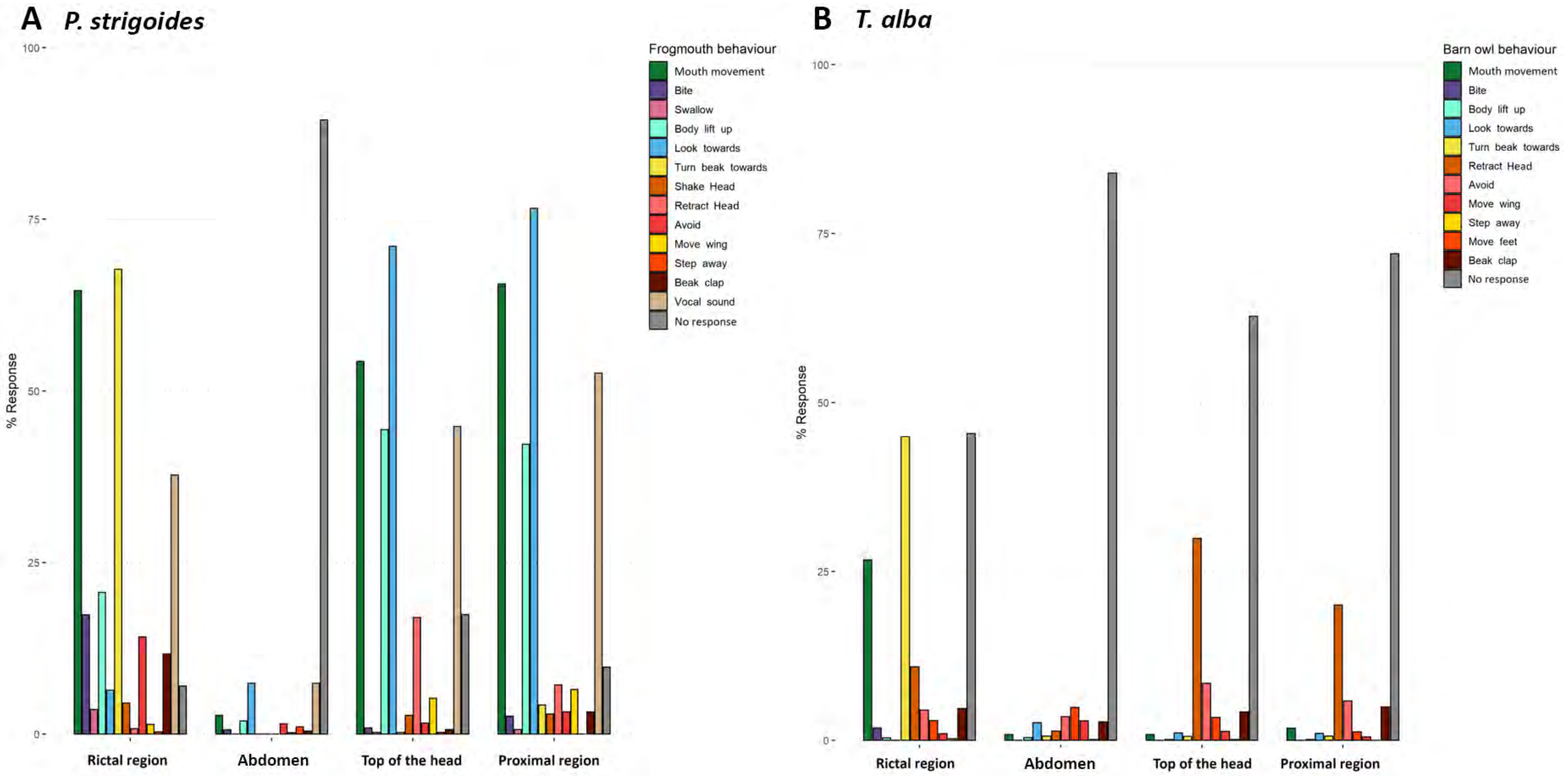


Figure 4-12. Full display of *Podargus strigoides* behavioural responses to touch on the four different touch point positions: rictal region, abdomen, head and proximal region. Bars represent the percentage of response of given behaviour to the number of touches made at each even touch point. Behavioural responses include: buccal movement, bite, swallow, body lift up, look towards, turn beak towards, shake head, retract head, avoid, move wing, step away, beak clap, vocal sound, and no response.

4.4 Discussion

Both *P. strigoides* and *T. alba* chicks developed in four plumage stages, however their development schedules differed. *P. strigoides* developed faster overall, yet their rictal bristles developed slower than *T. alba*'s. *P. strigoides* eyes also developed sooner and faster than *T. alba*. In *P. strigoides*, rictal bristles started emerging after the eyes were matured and were fully developed after the chick had fledged. In *T. alba* rictal bristles emerged alongside the development of the eye and were fully developed before the chick had fledged. In both species, rictal bristles are likely to be tactile in adults since Herbst corpuscles were found immediately surrounding their follicles. In addition, touches on the rictal region caused strong behavioural responses, especially feeding and avoidance behaviours.

Coincidence of rictal bristle emergence with developmental features

Rictal bristles developed faster in *T. alba* (taking 9 days from emergence to fully developed) than in *P. strigoides* (taking 12 days from emergence to fully developed) (Figure 4-5). In *T. alba* the full development of the rictal bristles was achieved from day 35, during the young juvenile phase, which coincided with the development of the feathers of the facial ruff and facial disk (Haresign and Moiseff, 1988). The facial ruff and disk play an important role in the acoustic abilities of the immature owls, including sound localisation accuracy, which is reduced until the ruff growth is complete (Knudsen et al., 1984; Haresign and Moiseff, 1988). Therefore, *T. alba* bristles could develop alongside hearing capabilities. It is not yet known when the hearing capabilities of *P. strigoides* reach adult levels.

In *T. alba* chicks the beak grows from day 1 to 12 beak growth rate decreases by half from day 13 to day 32, when the chicks reach fledgling size (Schönfeld and Girbig, 1975) and have mature rictal bristles (Figure 4-5). At 20 days old, *T. alba* chicks are also thermally emancipated (Taylor, 1994) and start tearing apart prey themselves (Durant and Handrich, 1998). Therefore, rictal bristles emerge once the chick is capable of thermally regulating and late in the development of the beak. The tactile sensitivity of the rictal region - i.e. development and presence of mechanoreceptors around the rictal bristle follicles – might coincide with the development of the beak and other facial feathers, such as the facial disk. Therefore, although rictal bristles and the tactile sensitivity of the rictal region does not appear to be functional in the early ages of the chick, they might well be later on. Unlike *T.*

alba, no previous research has studied the development of *P. strigoides* chicks and, therefore, there are no descriptions of other developing traits to compare to.

The development of the eyes occur faster in *P. strigoides*, and their eyes are mature at day 15 while *T. alba* eye maturation only ends at day 32 (Figure 4-5). Therefore, *P. strigoides* might rely more on vision than touch to get food as chicks, especially since their rictal bristles emerged a week after their eyes attained adult size. That rictal bristle emergence occurs after the maturation of the eyes might suggest that rictal bristles do not solely play a role in eye protection. Although, perhaps eye protection is not needed until after the chick has fledged and is foraging independently. Indeed, as *P. strigoides'* rictal bristle development is only complete after fledging, rictal bristles are likely to not play a major tactile role in developing *P. strigoides* chicks, and rather play a tactile role in the adult. Since *T. alba*'s rictal bristles developed faster (day 26 to day 35) than *P. strigoides* (day 23 to 35), and emerged fully before the eyes had attained their adult size (day 32), perhaps *T. alba* relies more on touch sensing in their younger juvenile phase. This also coincides with the period where the chick starts to feed independently on food brought back by the parents from day 20 (Durant and Handrich, 1998). Indeed, overall, the appearance of rictal bristles and the increase in behavioural responses coincide with independent feeding in both species. Investigating the emergence of rictal bristles in more species will help to test whether this association is consistent across birds with rictal bristles, and investigate if rictal bristles are functional in developing birds.

Chick development schedule

Both chick species remained in their nest while growing, were fed by their parents (Billerman et al., 2020), and both species chicks hatched with their eyes closed. Therefore, both species are not precocial and appear to be within the altricial spectrum. *T. alba* displayed a bare pink skin at hatching - a description that has been previously confirmed by Haresign and Moiseff (1988) - whereas *P. strigoides* had a white natal downy plumage at hatching. Therefore, *T. alba* have an altricial development type, while *P.* strigoides are semialtricial developmental type (2.4.1; Table 2-2; Starck and Ricklefs (1998)), which partially agrees with Bicudo et al. (2010)'s hypothesis that above-ground nesters tend to be more altricial than ground nesters, since *P. strigoides* hatchling development is slightly more advanced than *T. alba*.

Both chick species are altricial or semialtricial hatchlings, which suggests that functions such as temperature regulation, ground locomotion and flight only develop after birth (Ricklefs, 1973).The first stage of their development consisted of the development of their body feathers, which, might be related to the development of temperature regulation. The development of *P. strigoides* feathers described in this study was also faster than *T. alba*'s. *P. strigoides* nestlings have previously been identified as reaching half their adult mass in 30 days and fledging soon after (Körtner and Geiser, 1999b) - which fits with my observations here. *T. alba* nestlings attain their adult body mass around day 21 and fledge around day 40 (Pickwell, 1948; Schönfeld and Girbig, 1975; Wilson et al., 1986). This difference in growth could be related to their nesting ecology. Indeed open-nesting birds, such as *P. strigoides* (Billerman *et al.*, 2020), have a faster growth rate than hole-nesters, such as *T. alba* (Ricklefs, 1968).

The development of *T. alba* described in this study agrees with previous studies (Knudsen et al., 1984; Haresign and Moiseff, 1988), specifically that it takes 60 days for the chick to move from hatchling to fledgling, and within this time there are many changes in the presence and form of the facial feathers. Schönfeld and Girbig (1975) described the emergence and growth of the white down feathers (neoptiles) from day 1 to 10, and then the apparition of the grey/brown or yellow/brown feathers (mesoptiles) from day 12 to 15. These descriptions closely agree with the timeline of my study too. However, I observed the emergence of the mesoptiles slightly later than Schönfeld and Girbig (1975), at day 15. This difference could be due to the individual variation within the chicks and the non-continuous observation of chicks (every two to five days). Previous studies (Pickwell, 1948; Schönfeld and Girbig, 1975; Wilson et al., 1986) have reported that *T. alba* chicks are fully developed in size on the 21st day of their development, as they arrive at the peak of their body mass, and attain fledgling stage at 40 days old, although they were still moulting and shedding down feathers up until day 40 to 45. However, my observations differ as I noted that the eyes only attained their adult size around day 32 and the feathers were still moulting until 46 days old, therefore, placing these stages of development later on. However, my focus was on the development of their feather and the complete disappearance of their down feathers – which, as Schönfeld and Girbig (1975) state, are still shed until day 40 to 45 - and not their body mass. Furthermore, Haresign and Moiseff (1988) reported that the facial ruff continues to grow until 60-65 days along with the development of their ear canal. Therefore, although the chicks might be adult-sized around day 21, their full development is not yet complete.

Rictal bristle morphology and anatomy

The colour of the rictal bristles varied between the species. The bristles of *P. strigoides* were brown, to match their plumage, and similarly, *T. alba*'s white rictal bristles matched their white facial disk. Both species had their rictal bristle follicles anchored in the dermis of the skin with muscles fibres connecting the follicles together, which is consistent with previous studies (Ostmann et al., 1963; Homberger and De Silva, 2000; Delaunay, 2015) and my observations of other species in Chapter 3. *P. strigoides* and *T. alba* rictal bristles were different shapes - branched and unbranched, respectively, although they were of similar lengths. They also had a different number of Herbst corpuscles around their follicles (4 and 7, respectively) and a similar size of muscle bundles. This is consistent with the findings in Chapter 3, and further suggests that it is not possible to make predictions about follicle anatomy from bristle morphology. Both *P. strigoides* and *T. alba* possessed Herbst corpuscles in the immediate surroundings of their rictal bristles as adults, which indicates that their rictal bristles are likely to be sensitive to touch. As both species are altricial, tactile corpuscles observed in the adults are probably not fully developed at hatching and will only start developing from then (Páč and Malinovský, 1988). Subsequently, the rictal region is likely to become touch sensitive during the development of the chicks.

Chick behavioural responses to touch

The touch experiment in this study demonstrated that rictal region touches caused more occurrences of behavioural responses overall in both species, especially feeding behaviours (mouth movement and turns beak towards), compared to touches on any other region. Beak clapping especially increased following a rictal region touch as both species got older. This suggests that the rictal region is likely to be sensitive, and may become more sensitive as the chicks develop.

When touched with the cotton bud, *P. strigoides* chicks displayed vocal sounds throughout their development and lifted their body upwards while gaping their mouth wide, especially during the hatchling stage. These behaviours correspond to typical nestling behaviour: open mouth for food delivery, and sound and visual stimuli to attract the parents' attention (Gill, 2007). Vocalisations and body movements both play important roles in offspring-parent interactions and sibling competition (Roulin, 2004). *T. alba* chicks were vocally active while their box was open and when I touched their siblings with the cotton bud, but they did not vocalise directly as a response to touch. *T. alba* was generally more passive in the early stages of its development and more responsive as it developed, displaying avoidance and feeding behaviours once its rictal bristles started emerging. *P. strigoides* displayed feeding and avoidance behaviours throughout its development, even once its eyes opened, yet only an increase of beak clapping was significant once the rictal bristles started to emerge. Once *T. alba*'s eyes were opened, touches on the rictal region triggered more feeding and avoidance behaviours.

Conclusions

P. strigoides and *T. alba* are both altricial species that develop through four distinct stages, however, the timings of their development differ. Both species are born without rictal bristles and their emergence and growth do not follow the same pattern as their development stages as it was fastest in *T. alba*, which had the slowest developmental schedule. Both species have Herbst corpuscles present around the rictal bristle follicle as adults, and in both species, touches on the rictal region triggered feeding and avoidance behaviours, demonstrating that the region is likely to have tactile sensitivity. Full rictal bristle emergence and heightened behavioural responses to touch coincided with when the chicks started to feed independently. Therefore, I suggest that rictal bristles are unlikely to be functional in young chicks, but may play a role in feeding in older chicks and adults.

Chapter 5

The evolution of rictal bristles

Chapter summary:

The Class Aves underwent significant morphological modifications throughout their evolution, which led to the diversity of birds that we see today. Feathers are one of the most complex appendages that have arisen during avian evolution, and there are many different types that vary with function. Facial bristles, and especially rictal bristles, are the least described feather type and their presence or form have not yet been extensively and systematically recorded across avian species. Therefore, this study characterised rictal bristle presence and morphology (length and shape) over 1,022 avian species, including 91 families and 29 orders. The evolution of rictal bristles in the most recent common ancestor (MRCA) was also characterised here for the first time. Results revealed that rictal bristles were present in the MRCA of the avian phylogeny used here - their evolution underwent multiple events of disappearance and gain, and their morphology was most likely short bristles with barbs at the base. Other facial bristles, such as upper rictal, narial and interramal bristles, were also likely to have been present in the MRCA, while lorial and lower rictal bristles were absent. Rictal bristle presence and morphology were diverse between some orders, families and genera as well as within them, suggesting that phylogenetic relatedness is not the only driver of rictal bristle presence and morphology. Indeed, despite rictal bristle presence and morphology having a strong phylogenetic signal, they might also be influenced by life-history traits, such as those associated with foraging.

5.1 Introduction

Characterising the evolutionary history of discrete morphological characters helps us to understand trait evolution and function (Schultz et al., 2016). The Class Aves has undergone many significant morphological modifications throughout their evolution (Mayr, 2016), leading to an enormous diversity of traits amongst the >18,000 known bird species (Barrowclough *et al.*, 2016). Amongst those traits, beak morphology is probably one the most variable characters, which has evolved multiple times. Beak morphology has been suggested to have evolved to resist feeding forces, facilitate successful food manipulation and song production (Soons et al., 2015). Indeed, beak shape, keratin thickness and aspect ratio (depth and width relative to length), all vary between species depending on their feeding strategies (Soons et al., 2015). Avian feather morphology is another traits that undergone morphological modifications throughout their evolution, e.g. from a unbranched, hollow, tubular feather to a vane feather (Prum and Brush, 2002). Indeed, avian feathers are perhaps one of the most complex morphological structures that have evolved in animals (Chuong et al., 2000; Prum and Brush, 2002) and vary in shape, size, branching (presence of barbs and barbules on the rachis) and function (Stettenheim, 2000; Yu et al., 2004). Even facial bristles - feather-like structures - present on the rictal, lorial, narial and interramal regions, exhibit diverse structural characteristics. They can be relatively stiff, tapered, hair-like feathers or semi-bristles, which are softer bristles with barbs, or sometimes with barbules at the base or on most of the length of their rachis (Figure 5-1) (Gill, 2007; Pass, 1989). Avian rictal bristles are present just above the tomium (edge of the upper beak) of many nocturnal and diurnal species (Chapter 2, Figure 2-4), such as Apterygidae, Caprimulgidae, Aegothelidae, Tyrannidae, Mimidae, and Accipitridae (Lederer, 1972). Despite this, the presence of rictal bristles has not been extensively and systematically recorded across species. Moreover, little is known regarding the plesiomorphy (ancestral character state) of rictal bristles so its origin and evolution within birds is unknown.

This study will address the following hypothesis outlined in Chapter 2:

- **Rictal bristles will be present in a common avian ancestor**

As early-feathers are thought to have been simple bristles, it would be expected that the plesiomorphy of rictal feathers in the most recent common ancestor (MRCA) of birds might have been bristles.

- **Rictal bristle presence and form will vary within species, families and order**

Avian phylogeny has undergone many morphological modifications throughout evolution, therefore several events of rictal bristle disappearance and gain would be expected.

This study will determine the plesiomorphy and distribution of rictal bristle presence and morphology (length and shape) across a large number of avian species, as well as in some detail in a small number of selected clades, i.e. Palaeognathae as it is the oldest clade and the clade of Caprimulgiformae and Passeriformes as they are diverse and contain more nocturnal and diurnal species, respectively. This study will also determine if these traits evolved independently from the phylogeny by assessing their phylogenetic signal (Pagel's λ) and if the different locations of other facial bristles evolved alongside the rictal bristles. Finally, the possibility of rictal bristles being a homologous structure will be discussed.

5.2 Material and methods

5.2.1 Samples and measurement

Rictal bristle presence/absence and morphology were described and measured from 1,022 avian species (~10% recorded species), representing 418 genera, from 91 families (37% recorded families) and 29 orders (73% of all orders). Specimens examined were predominantly from the skin collection of the World Museum of Liverpool, Manchester Museum and Wollaton Hall Museum in Nottingham, all situated in the United Kingdom. Only the best-preserved specimens were chosen for this study to ensure accurate measurements of bristle length and shape, which would not have been altered by the process of skin removal and specimen conservation. Where possible, two specimens per species were measured for 82% of all species examined. Attempts were also made to measure adult specimens of each sex; however, this was not always possible, since some specimens were either not labelled, or incorrectly labelled, resulting in 274 species for which each sex was measured, 50 and 31 species for which specimens were of the same sex (males, females, respectively), 141 and 84 species for which one of the specimens had one sex labelled and one unknown (males, females, respectively). A total of 374 species were measured from individuals of unknown sex, and a total of 412 females were measured and 508 male specimens were measured. Rictal bristle presence, normalised length and shape were compared between males and females using a non-parametric Mann-Whitney U Test (for presence and normalised length) and Chi-square Test (for shape), since the data was not normally distributed and skewed. There was no difference in the presence (N=554, Mann-Whitney U test, W=38365, *p*=1), normalised length (N=554, Mann-Whitney U test, W=4518, *p*=0.82) and shape (N=554, Chi-square test, X-square=0.12, df=2, *p*=0.94) of rictal bristles recorded between sexes of the species measured in this study, therefore, rictal bristles are likely to be sexually monomorphic.

Facial bristles were initially identified by sight and touch in each specimen as tapered, hair-like feathers, which felt stiffer than, and often protruding above, the surrounding plumage feathers. Confirmation of their presence came from identifying their location on the upper rictus, lores, lower rictus, nares and interramus regions (Figure 5-5F). For each specimen, bristles were recorded as either present or absent from each of these locations. The term rictal bristle is described here as bristles on both the upper rictal and/or the lorial region, as there was no clear differentiation and morphological differences between the bristles found in these regions. While bristles on the upper rictus were present on all species that possessed rictal bristles, it was not true for lorial bristles. Hence, upper rictal and lorial bristles were included separately in the bristle location analyses but were combined for all rictal bristle analyses. Where present, rictal bristle shape was recorded as: i) unbranched rictal bristles, ii) rictal bristles with barbs only at the base, iii) branched rictal bristles, i.e. barbs and barbules present on most of the length of the bristle rachis (Figure 5-1). In addition, the three longest rictal bristles were measured on both sides of the head of each specimen using digital callipers, and these lengths were averaged to provide a mean length of rictal bristles per species. The mean bristle length per species was then normalised by dividing the mean length by the body mass of the species giving a normalised length (with no associated units). Information on avian body mass was taken from the Eltontraits 1.0 dataset (Wilman *et al.*, 2014). Body mass estimates for species not present in Eltontraits were taken from the CRC handbook of avian body masses (Dunning Jr., 2007). Normalised rictal bristle length was included as a continuous variable in all subsequent analyses. However, in order to qualitatively discuss the results, the normalised bristle length was considered in three categories: short (<0.225), medium (0.225-0.67) and long (>0.67).

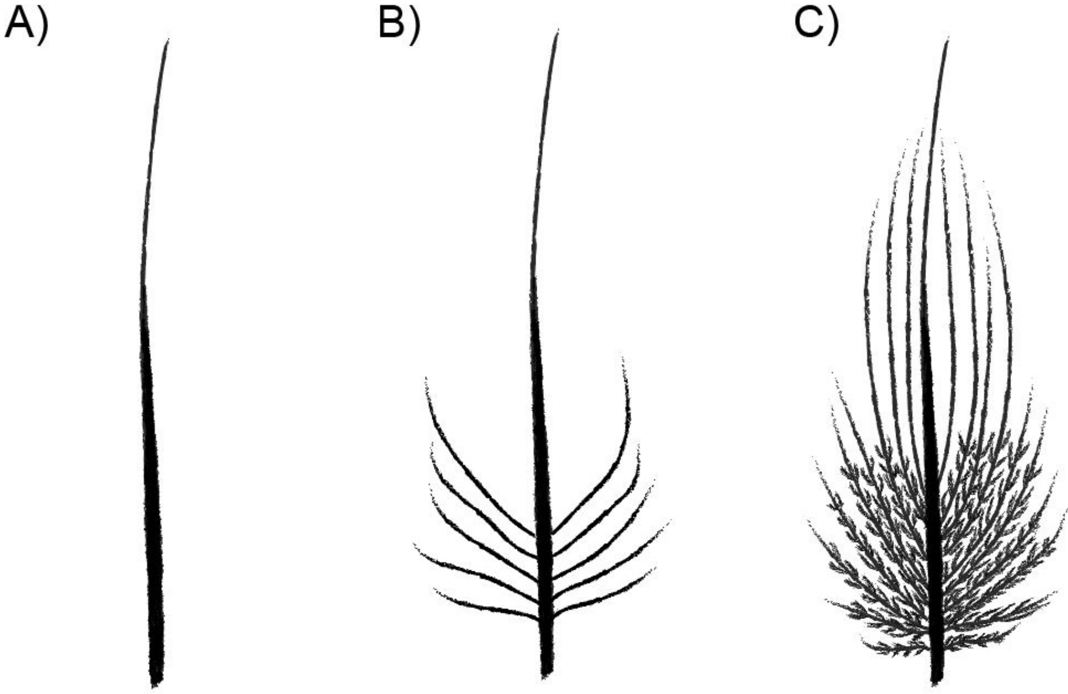


Figure 5-1. Diagram of the different facial bristle shapes: **A)** Unbranched bristles, **B)** Bristles with barbs at the base, and **C)** branched rictal bristles, i.e. barbs and barbules present on most of the length of the bristle rachis.

5.2.2 Bristle plesiomorphy reconstruction using stochastic character traits

Ancestral reconstruction of rictal bristle presence/absence character

Following Felice *et al.* (2019), a single consensus phylogenetic tree was generated from the Hackett posterior distribution of trees from Birdtree.org (Jetz *et al.*, 2012) with a sample size of 10,000 post burn-in, using the TreeAnnotator utility in BEAST software (Bouckaert *et al.*, 2019) with a burn-in of 0. Maximum Clade Credibility (MCC) with the option "*-heights ca*" was selected as the method of reconstruction. The common ancestor trees option (-*heights ca*) builds a consensus tree by summarising clade ages across all posterior trees; this option was chosen over a simple MCC , which can result in negative branches when the subset of trees is low in frequency since it averages node heights across different subsets of posterior trees for each clade. Both the consensus tree and posterior distribution of 10,000 trees were imported into R Studio v. 1.2.5 (R Core team, 2018) and pruned so that only species present in the dataset of this study remained in the phylogeny. Taxon names were modified where necessary to match those from the Birdtree.org (http://birdtree.org) species record. The root age of the basal node of the consensus tree was calculated using the function *tree.max* from the FossilSim package (Barido-Sottani *et al.*, 2019).

The ancestral state reconstruction (ASR) of the presence/absence character was conducted using the function *make.simmap* (package phytools v0.7-70) (Revell, 2012), which simulated stochastic character mapping by using the binary character presence/absence of rictal bristles on the consensus tree (nsim=10,000), with the results summarised by using the function *describe.simmap* (phytools). Stochastic mapping accounts for uncertainty in the reconstruction of the ancestral states, by allowing characters to change multiple times along a single branch (Nielsen, 2002). Three commonly-used evolutionary transition rate models - equal-rates (ER), symmetrical (SYM) and all-rates-different (ARD) - were evaluated across the posterior distribution of 10,000 trees using the ace function in *ape* v5.4-1 package (Paradis and Schliep, 2019). For the ER model, all transitions rates were governed by a single parameter; for the SYM model, transitions 0->1 and 1->0 occur at the same rate but may differ from the transition rate between state 1 and 2; and in ARD model, each rate referred to a different parameter. Model fits were evaluated using the *fitDiscrete* function in the R package *geiger* v2.07 (Pennell *et al.*, 2014). Model fits were determined using the AIC values (Akaike Information Criterion) and AIC weight (AIC.w)(Akaike, 1973, 1974, 1978; Burnham and Anderson, 2002). AIC estimates the quality of each model in comparison to the other models, i.e. relative model fit and AIC.w identify which models have the greatest strength. The phylogenetic signal of the rictal bristle presence character was examined using the *phylosig* function in the R package *phytools*; a strong phylogenetic signal indicated that the trait is evolving by Brownian motion, as indicated by a lambda value close to 1 and $P < 0.05$, and opposed to a lambda value= 0, which signifies that the character has no phylogenetic signal on to the phylogeny. The *densitymap* function (*phytools*) was used to plot the consensus tree onto which the posterior density of rictal bristle presence/absence was mapped. The mapped value was the probability of having rictal bristles present. Adobe Photoshop CC 2018 software (Adobe Systems Incorporated, San Jose, California) was used to customise the resulting radial tree.

Ancestral reconstruction of two rictal bristle morphological characters

Using a subset of the data corresponding to only species possessing rictal bristles, an ancestral state reconstruction analysis was conducted on rictal bristle normalised length to body mass using the *contMap* function in the R package *phytool* (Revell, 2012) and on rictal bristle shape using *make.simmap* (nsim=10,000). The *contMap* function reconstructs ancestral character states at each node using the *fastAnc* function and interpolates change along branches. Both resulting trees were plotted in RStudio and customised using Photoshop. The best fitting model was determined using *fitContinuous* in the R package geiger v2.07 (Pennell *et al.*, 2014) for the normalised rictal bristle length character and *fitDiscrete* (Geiger package) for the bristle shape character. Brownian motion (BM), Ornstein-Uhlenbeck (OU) and Early-burst (EB) models were compared using AIC values obtained with *fitContinuous* data while AIC values of ER, SYM and ARD models were compared in *fitDiscrete*. The Brownian motion model assumes that the correlation structure between trait values and shared ancestry for pairs of species is proportional, while for the EB model, the rate of the evolution increases or decreases exponentially through time, and the OU model, the continuous character is assumed to evolve towards a fitness optimum. The phylogenetic signal of both normalised length and shape characters were examined using the *phylosig* function (phytools package)

Ancestral reconstruction of the presence character at each facial bristle locations

The same ancestral state reconstruction procedure for the rictal bristle presence character was conducted for each facial bristle locations: upper rictal, lorial, lower rictal, narial, interramal bristles (Figure 5-5**Figure 5-**F) for 10,000 simulations of randomly sampled stochastically mapped trees. The resulting tree for each of the facial bristle locations was plotted in RStudio. The best fitting transition rate model to each facial bristle location was determined using the *fitDiscrete* function in the R package *geiger* v1.3-1 (Pennell *et al.*, 2014). The phylogenetic signal of bristle presence at each location was examined using the *phylosig* function (package *phytools*) (Revell, 2012).

Sensitivity analyses

The robustness of the ancestral state reconstruction findings for rictal bristle presence, morphology and locations was evaluated by testing for bias within the datasets and in each *make.simmap* analysis (as per e.g. Losos, 1994; Donoghue and Ackerly, 1996). As a consensus tree was used to conduct the ancestral state reconstruction of the rictal bristle presence, 100 trees were randomly sampled from the posterior distribution and used throughout the study as a way to account for uncertainty in tree topology and branch length in the ancestral state reconstruction analysis. For each of these 100 trees, 100 character histories were randomly sampled, generating 10,000 character maps, to account for the uncertainty associated with tree topology, branch length and timing of the transitions between morphological states. To evaluate sampling bias, a probability of oversampled or undersampled families was calculated to weigh the likelihood of a given bird species. The probability was subsequently used in the weighted analysis by downsampling the datasets (full dataset, and presence only subset) to 70%, 80% and 90% of the species, removing families that have a sampled bias (as per Schultz et al., 2016). The stochastic mapping procedure was then repeated 10,000 times, with the mean number of transitions, gains and losses calculated, as well as the average time the character spent in each state, for each of the subsamples (70, 80, 90%).

5.2.3 Patterns of gains and losses across the phylogeny of selected clades

To qualitatively investigate the evolutionary gains and losses of rictal bristles, three clades of interest were selected: the Palaeognathae, Caprimulgiformae, and the Passeriformes. Palaeognathae were chosen since they represent the most ancient lineage; Caprimulgiformae were selected as they represent a highly diverse order, dominated by nocturnal species, and Passeriformes as it is the largest and most species-rich avian order, as well as being composed of predominantly diurnal species. All figures were plotted with RStudio and customised with Photoshop.

5.3 Results

5.3.1 Bristle plesiomorphy reconstruction of stochastic character traits

Ancestral reconstruction of the rictal bristle presence/absence character

The comparison between different transition rate models revealed that the equal rate (ER) model was rejected in favour of a more parametrised all rates different (ARD) model (Table 5-1). The analysis of 10,000 simulations of stochastic character mapping reported an average of 63 changes between character states across the avian phylogeny, of which an average of 16 state changes was in favour of a rictal bristle gain and an average of 47 character state changes were towards a loss of rictal bristles (Figure 5-2). Overall, rictal bristles were absent in 61% of the evolutionary period covered by the tree in this study, and in 656 species of the 1,022 measured species. Just over a third of species (n=366) had bristles present. Despite rictal bristle absence being more common, the ancestral character state reconstruction analysis revealed that the presence of rictal bristles was the most likely plesiomorphy state, with an 87% likelihood of being reconstructed at the basal node of the phylogeny (Figure 5-2). Rictal bristle presence had a strongly significant phylogenetic signal (phylosig, lambda=0.89, $P<0.001$). Rictal bristles were notably present in Ratites, Caprimulgiformae and Passeriformes (Figure 5-2).

Table 5-1. Comparison between the different transition rate models using AIC (Akaike Information Criterion) **values and weight**: equal-rates (ER), symmetrical (SYM) and all-rates-different (ARD) models for categorical data and Brownian motion (BM), Ornstein-Uhlenbeck (OU) and Early-burst (EB) models for continuous data. Values in bold illustrate the best fit model chosen for the analysis.

Character	Model	AIC values	AIC weight
Rictal bristle	ER	455.2	0.00025
	SYM	455.2	0.00025
	ARD	438.6	**0.99950**
Normalised length	**BM**	-886.8	**0.44528**
	OU	-884.8	0.16095
	ER	-886.6	0.39377
Shape	ER	279.8	0.00006
	SYM	282.7	0.00001
	ARD	260.2	**0.99993**
Upper rictus	ER	455.2	0.00025
	SYM	455.2	0.00025
	ARD	438.6	**0.99950**
Lores	**ER**	534.1	**0.42226**
	SYM	534.1	0.42226
	ARD	536.1	0.15548
Lower rictus	ER	673.8	0
	SYM	673.8	0
	ARD	581.2	**1**
Nares	**ER**	516.4	**0.41388**
	SYM	516.4	0.41388
	ARD	518.1	0.17225
Interramus	ER	573.2	0.00019
	SYM	573.2	0.00019
	ARD	556.1	**0.99962**

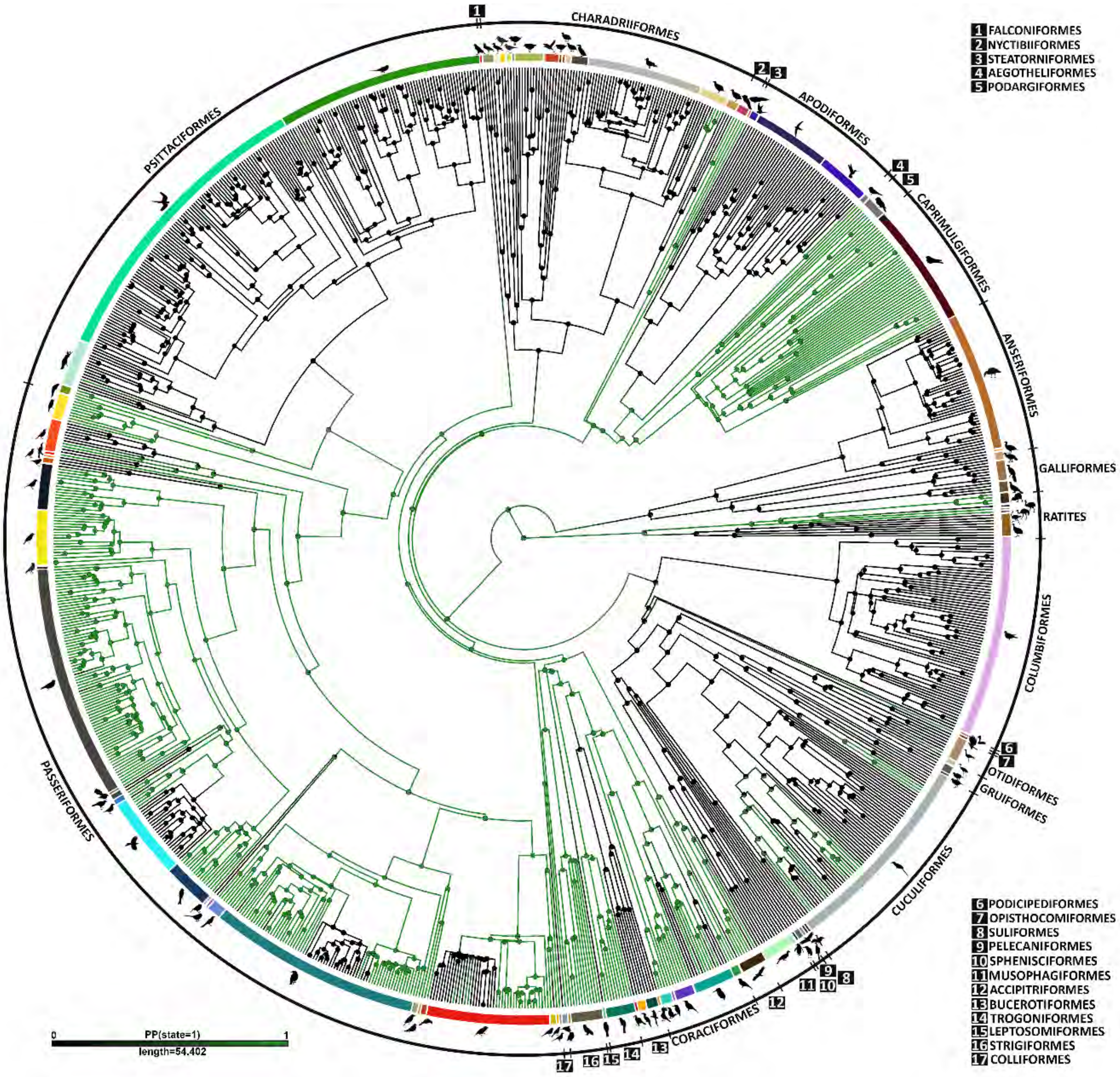


Figure 5-2. **Resulting phylogenetic tree mapping the ancestral character reconstruction of rictal bristle occurrence from 10,000 stochastic character maps**. Terminal branches correspond to the different species measured. The colour of the branch edges in the tree gives the posterior probability of the rictal bristle character through avian evolution. Black indicates the high probability of rictal bristle absence while green indicates their presence. Ancestral state appears to be the presence of rictal bristles. The inner coloured arc encircling the radial phylogenetic tree with bird silhouette illustrates the different avian families, which each species belongs to, and the black outer circle correspond to the avian orders.

Ancestral reconstruction of two rictal bristle morphological characters

i. Normalised rictal bristle length

The model with the best fit to normalised bristle length was Brownian motion as its AIC weight showed the greatest strength (Table 5-1). Normalised bristle length also had a strong significant phylogenetic signal (phylosig, lambda=1, *P*<0.001). Normalised rictal bristle length was mapped onto a phylogenetic tree as a continuous trait, and ranged from 0.003913 to 0.913882. The ancestral state reconstruction suggested that short normalised rictal bristle lengths (<0.225) were the most likely ancestral state (within the 95% confidence interval) (Figure 5-3). Most species (~83%) had relatively short normalised rictal bristle lengths (<0.225). Variation in normalised rictal bristle length appeared in the Passeriformes order and Caprimulgiformae, suggesting that normalised rictal bristle length increased through time in these orders (Figure 5-3). Medium normalised rictal bristle lengths (0.225<length<0.67) were seen in 12% of the species in these clades, and more precisely in Aegothelidae (both species), Caprimulgidae (71% of the family, n=30), Podargidae (29%, n=2), Dicruridae (69%, n=11), Platylophidae, Rhipiduridae and Tyrannidae (44%, n=4). Only two species had long normalised rictal bristles (>0.67; yellow-green in Figure 5-3A), one in each aforementioned family (*Uropsalis segmentata* for the Caprimulgidae and *Dicrurus atripennis* for the Dicruridae). A decrease in normalised rictal bristle length through time also occurred in Passeriformes and Caprimulgiformae (return to dark blue in Figure 5-3).

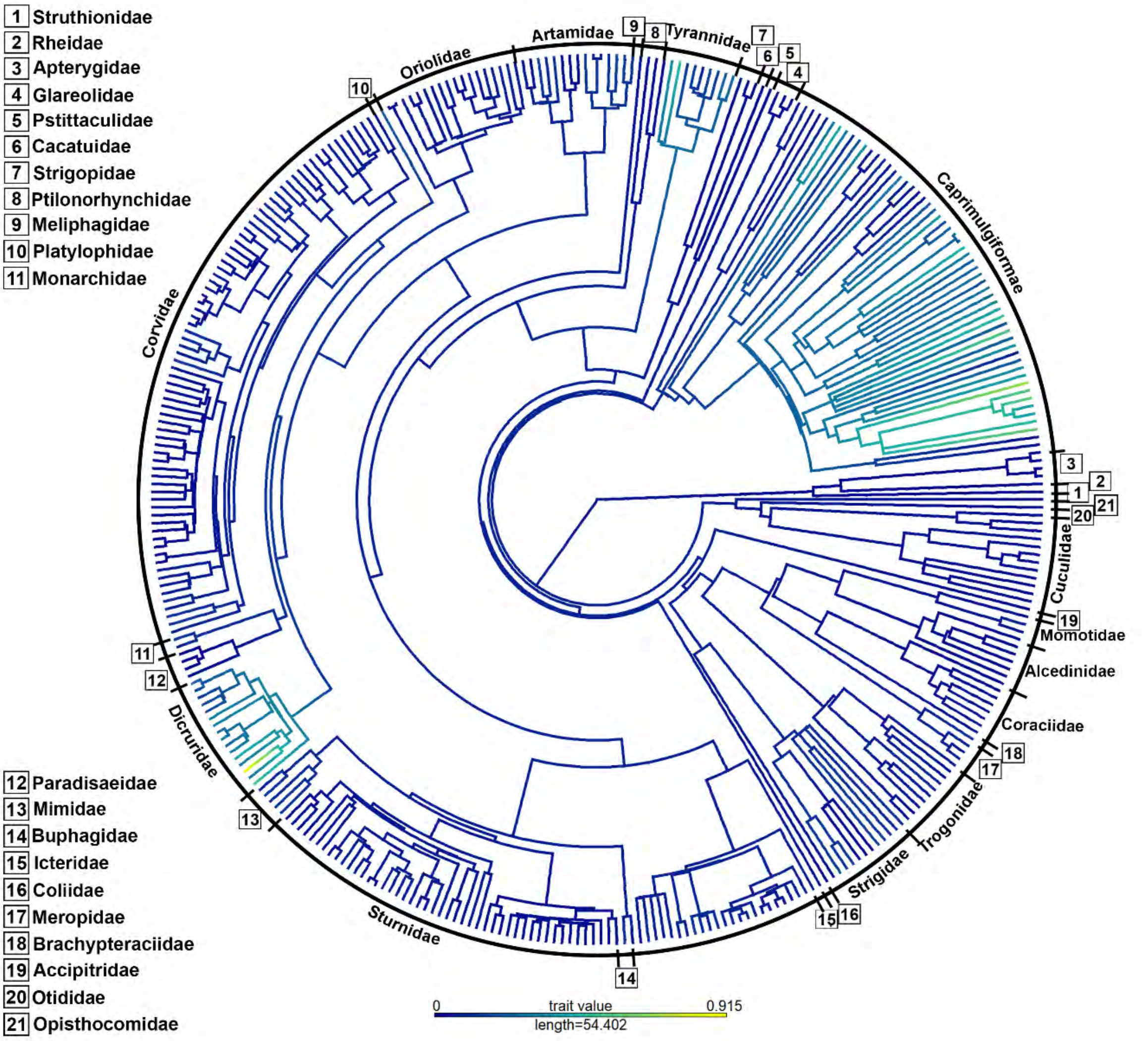


Figure 5-3. Phylogenetic trees used for ancestral state reconstructions of normalised rictal bristle length. A gradient dark blue to yellow illustrated the normalised length of rictal bristles from short to long.

ii. Rictal bristle shape

The model that best fitted the rictal bristle shape was ARD as its AIC weight had the greatest strength (Table 5-1). The analysis of 10,000 simulations of stochastic character mapping revealed the rictal bristle shape - barbs at the base - was the most likely ancestral state (86% confidence in ASR), and reported an average of 62 changes between shape stochastic states across the avian phylogeny (Figure 5-4). Most of these changes occurred between rictal bristles with barbs at the base to unbranched rictal bristles states (n=31), followed by changes from rictal bristles with barbs at the base to branched rictal bristle (n=15) (Table 5-2). The stochastic state, unbranched rictal bristle shape, spent the longest period of the evolutionary time covered by the phylogeny of this study (63%). The average time spent in the base branched state was 17% and in the branched state was 21%. Overall, the most predominant rictal bristle shape was unbranched rictal bristles (for n=292, i.e.

80% of all species measured), followed by branched rictal bristles (n=47, i.e. 13% of all species measured). Despite being the likely ancestral state in the MRCA, rictal bristles with barbs at the base were present in only 7% of the species measured (n=26) in this study (Figure 5-4).

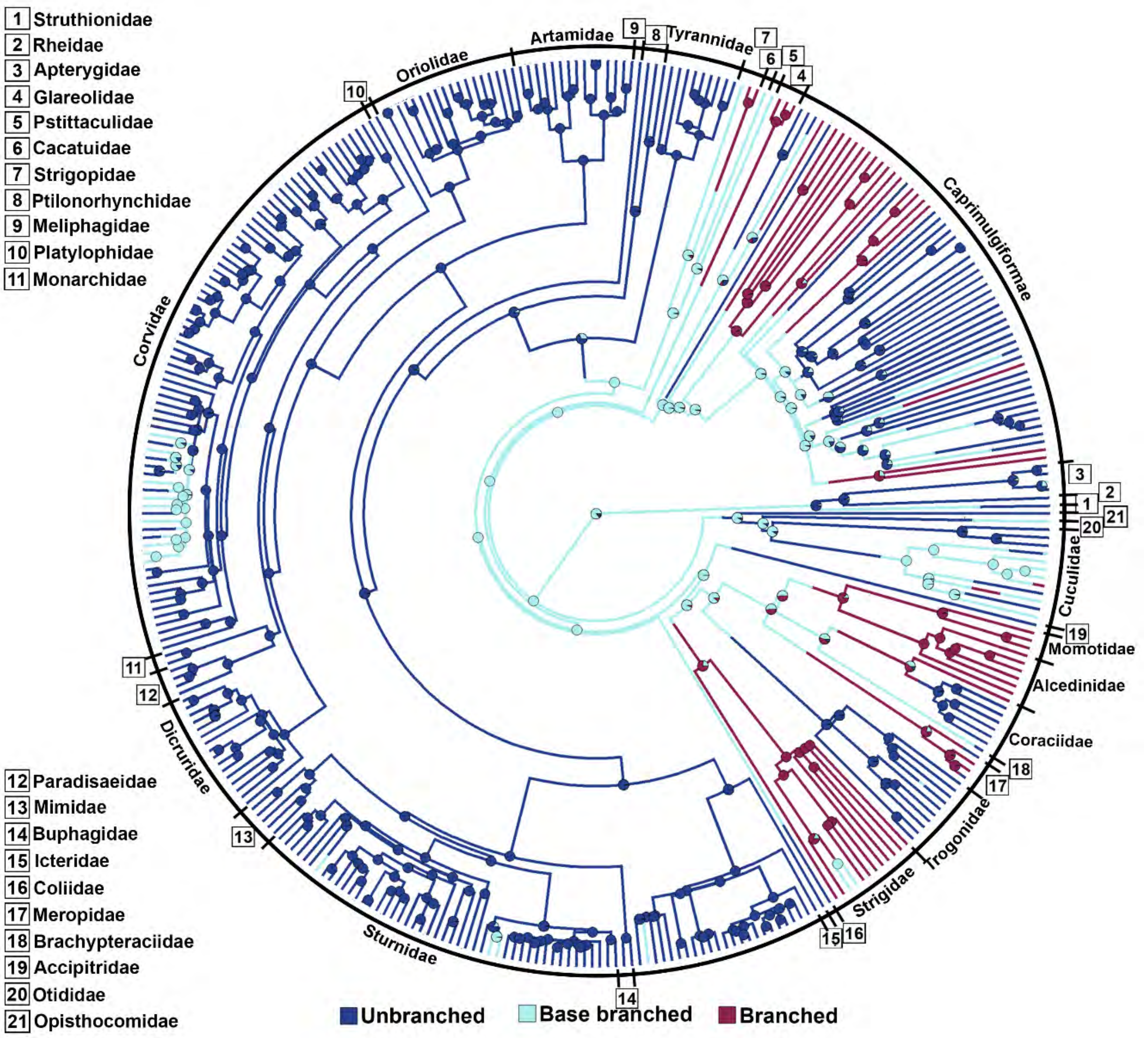


Figure 5-4. Phylogenetic trees used for ancestral state reconstructions of rictal bristle shape. Dark blue represented unbranched rictal bristles, light blue represented rictal bristle with barbs at the base, and dark magenta represented branched rictal bristles (barbs and barbules on most of the bristle length).

Table 5-2. Average of changes from a state to another reported by the stochastic character state mapping (nsim=10,000) **of the rictal bristle shape on the consensus tree analysis, and the sensibility analysis for shape**: on the 100 trees randomly sampled from the posterior distribution, and for the downsampling analysis (90%, 80%, and 70% subsampled dataset) mapped on to the consensus tree. Rictal bristle shape included: i) Unbranched rictal bristle (Unbr), ii) Rictal bristle with barbs at the base (Base), and iii) Branched rictal bristles (Br).

Trees / Changes (x → y)	Average of n changes	Base→Br	Base→Unbr	Br→ Base	Br→Unbr	Unbr→ Base	Unbr→Br	Time spent in ancestral character	% confidence in ASR
Consensus tree	62	15	30	3	4	10	0	Base 17%	87%
Random trees	54	4	15	7	15	12	1	Branched 32%	75%
90%	59	13	32	2	0	9	3	Base 18%	85%
80%	56	14	28	3	0	10	1	Base 17%	87%
70%	42	4	5	4	19	10	0	Branched 34%	67%

Ancestral reconstruction of the presence character at each facial bristle locations

Collectively across all species measured facial bristles were located on the upper rictus and lores (termed rictal bristles), as well as the lower rictus, nares and interramus regions (Figure 5-5). When rictal bristles were present (in 36% of the species measured, n=366), they were always present on the upper rictus region, whereas lorial bristles were less common (found in only 20% of all species measured, n=206), and only occurred in species that already had bristles on the upper rictus (Figure 5-5A, B). Comparisons between transition rate models for each facial bristle location resulted in favour of the ARD model for the upper rictus, lower rictus and interramus data, while ER models were selected for the lores and nares data (Table 5-1; Figure 5-6 to10). Stochastic character state mapping reported multiple changes between states across the avian phylogeny, of which a higher number of changes were in favour of a gain of lorial and narial bristles, and a loss of upper and lower rictal, and interramal bristles (Table 5-3; Figure 5-6 to 10). All facial bristle locations were absent for more of the time during the evolutionary period covered by the tree (Table 5-3; Figure 5-5) than when they were present. Despite this, the ancestral state reconstruction analysis revealed that the presence of upper and lower rictal, and interramal bristles was the most likely ancestral state for species measured with 87%, 100% and 97% confidence in ASR respectively (Figure 5-5, 6, 8, 10); whereas absence was the most likely ancestral state character for lorial and narial bristles (99% and 98% confidence in ASR respectively; Figure 5-7, 9). Each facial bristle location had a strong significant phylogenetic signal (Table 5-3; phylosig, $\lambda > 0.70$, $p < 0.001$).

Within the ratites, Caprimulgiformae, Strigiformes, Trogoniformes, Strigopidae (Psittaciformes), and Glareolidae (Charadriiformes), all measured species possessing rictal bristles had both upper rictal and lorial bristles, whereas, in the Passeriformes, lorial bristles did not always coincide with the presence of upper rictal bristles (Figure 5-5A, B, 6, 7) and their presence seemed to vary (n=89, i.e. 28%; Figure 5-5A, B, 7). Bristles on the lower rictus were predominant in 55% of all Passeriformes species measured, as well as present in all Strigopidae, Strigiformes, Trogoniformes, Momotidae and Numididae species, and in some Coraciidae, Cuculidae and Cracidae; but most Caprimulgiformae species did not present any bristles on the lower rictus, with the noticeable exceptions of Podargidae and Nyctibiidae (Figure 5-5C and 8). Most species with rictal bristles also had bristles on the nares (67 %, n=245) and interramus (79%, n=289) regions (Figure 5-2, 5D, E, 9, 10).

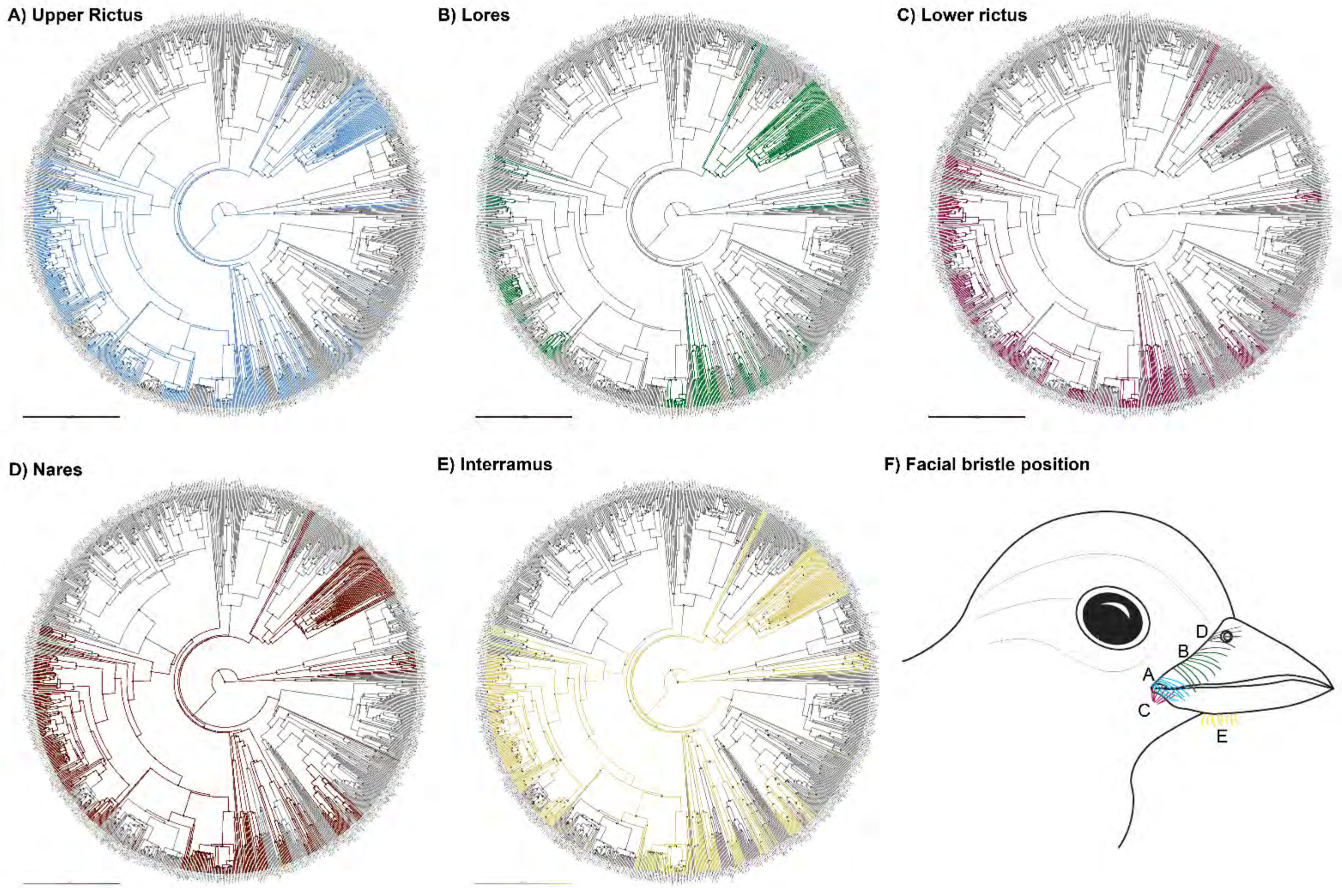


Figure 5-5. Comparison of the ancestral character estimation for the presence of A) Upper rictal, B) Lorial, C) Lower rictal, D) Narial, E) Interramal bristles, and F) Schematic drawing of a bird's head illustrating the position of the different type of facial bristles around the beak. Terminal branches correspond to the different species measured. The colour of the branch edges in the tree gives the posterior probability of the rictal bristle character through avian evolution, grey indicates the high probability of the different facial bristle absence while colours indicate the presence of these facial bristles.

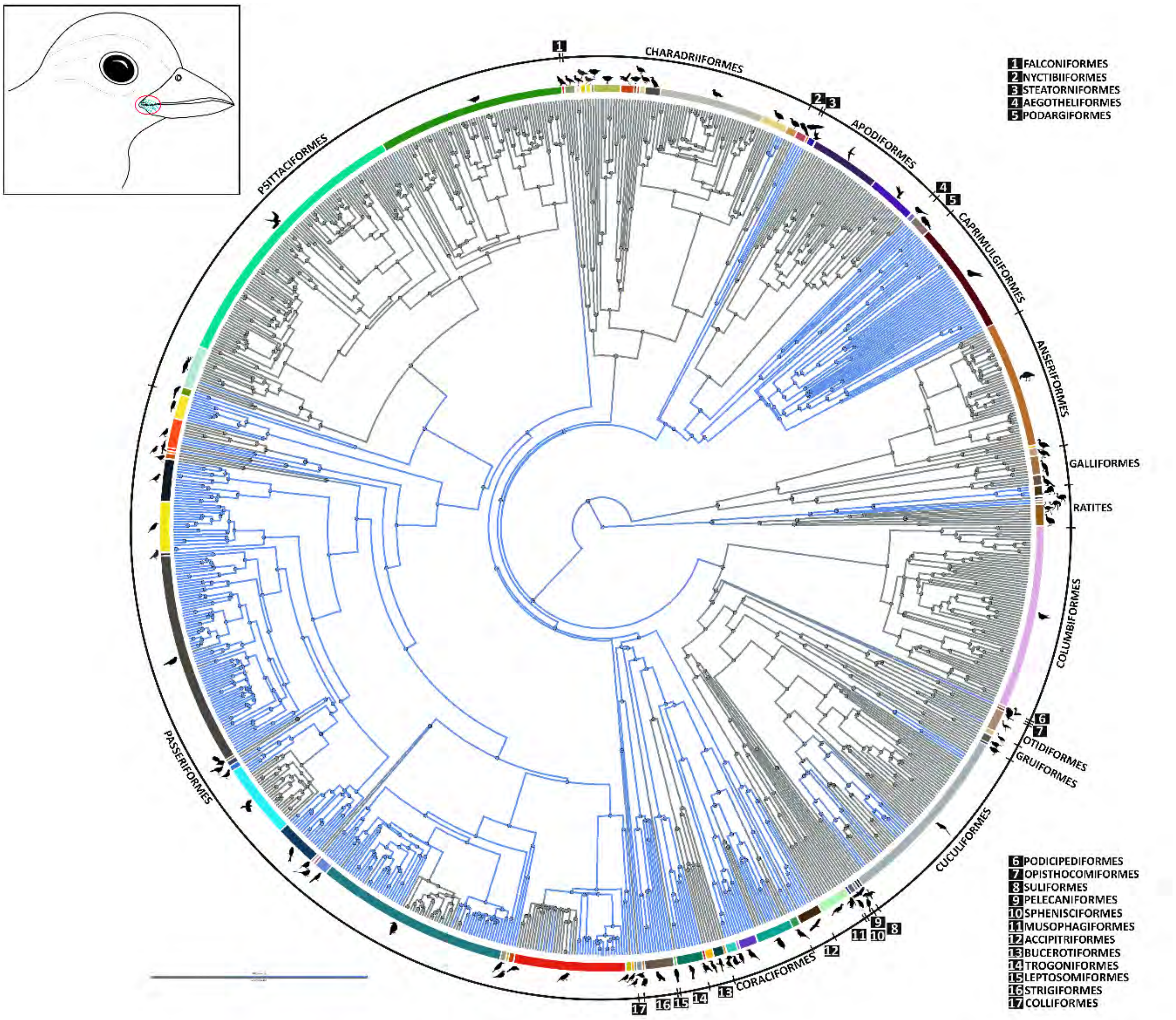


Figure 5-6. Zoomed in phylogenetic tree mapping the ancestral character estimation for the presence of upper rictal bristles. Terminal branches correspond to the different species measured; blue branches indicates the high probability of the presence while grey, the absence. The inner coloured arc encircling the radial phylogenetic tree with bird silhouette illustrates the avian family each species belongs to, and the black outer circle correspond to the avian orders.

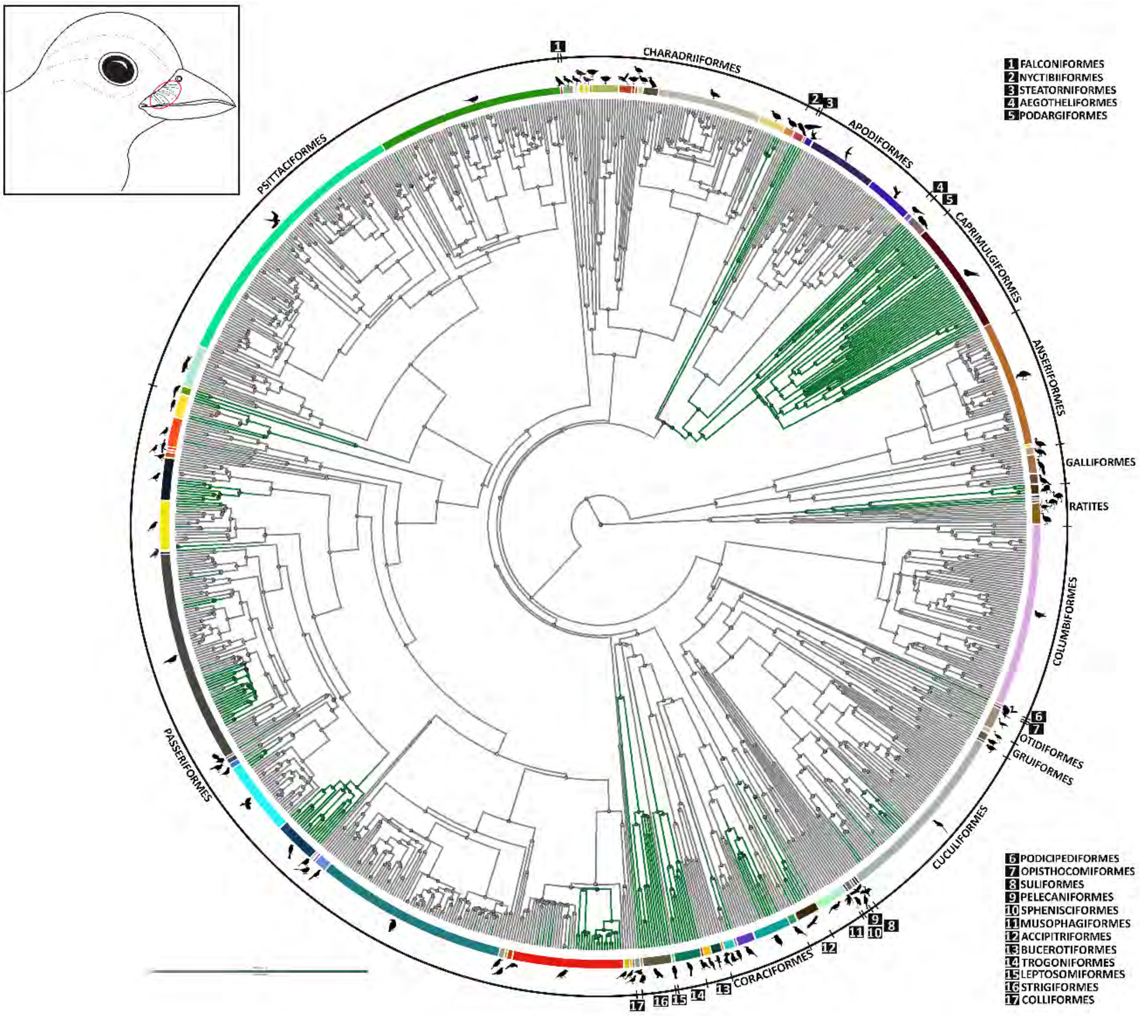


Figure 5-7. Zoomed in phylogenetic tree mapping the ancestral character estimation for the presence of lorial bristles. Terminal branches correspond to the different species measured; green branches indicates the high probability of the presence while grey, the absence. The inner coloured arc encircling the radial phylogenetic tree with bird silhouette illustrates the avian family each species belongs to, and the black outer circle correspond to the avian orders.

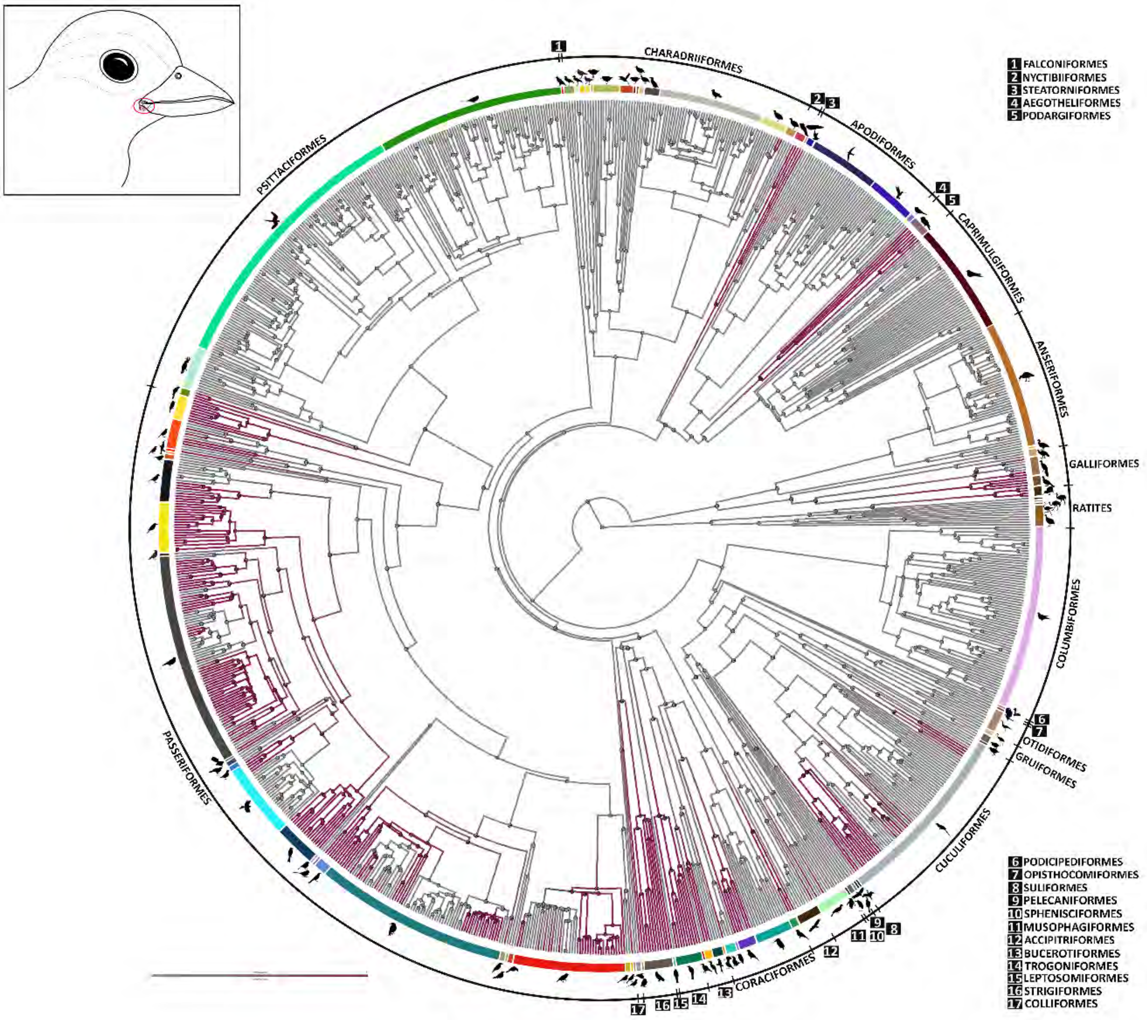


Figure 5-8. Zoomed in phylogenetic tree mapping the ancestral character estimation for the presence of lower rictal bristles. Terminal branches correspond to the different species measured; purple branches indicates the high probability of the presence while grey, the absence. The inner coloured arc encircling the radial phylogenetic tree with bird silhouette illustrates the avian family each species belongs to, and the black outer circle correspond to the avian orders.

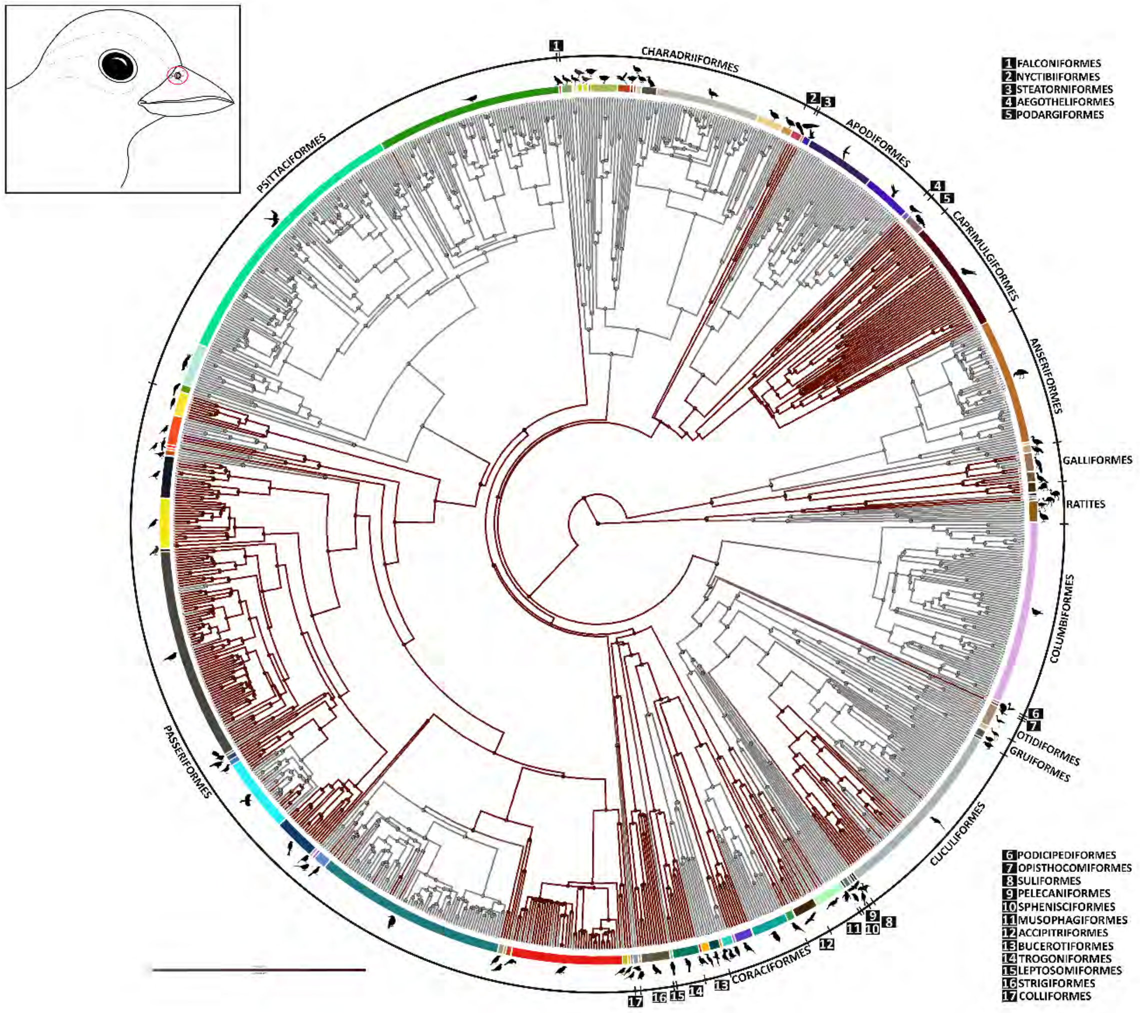


Figure 5-9. Zoomed in phylogenetic tree mapping the ancestral character estimation for the presence of narial bristles. Terminal branches correspond to the different species measured; brown branches indicates the high probability of the presence while grey, the absence. The inner coloured arc encircling the radial phylogenetic tree with bird silhouette illustrates the avian family each species belongs to, and the black outer circle correspond to the avian orders.

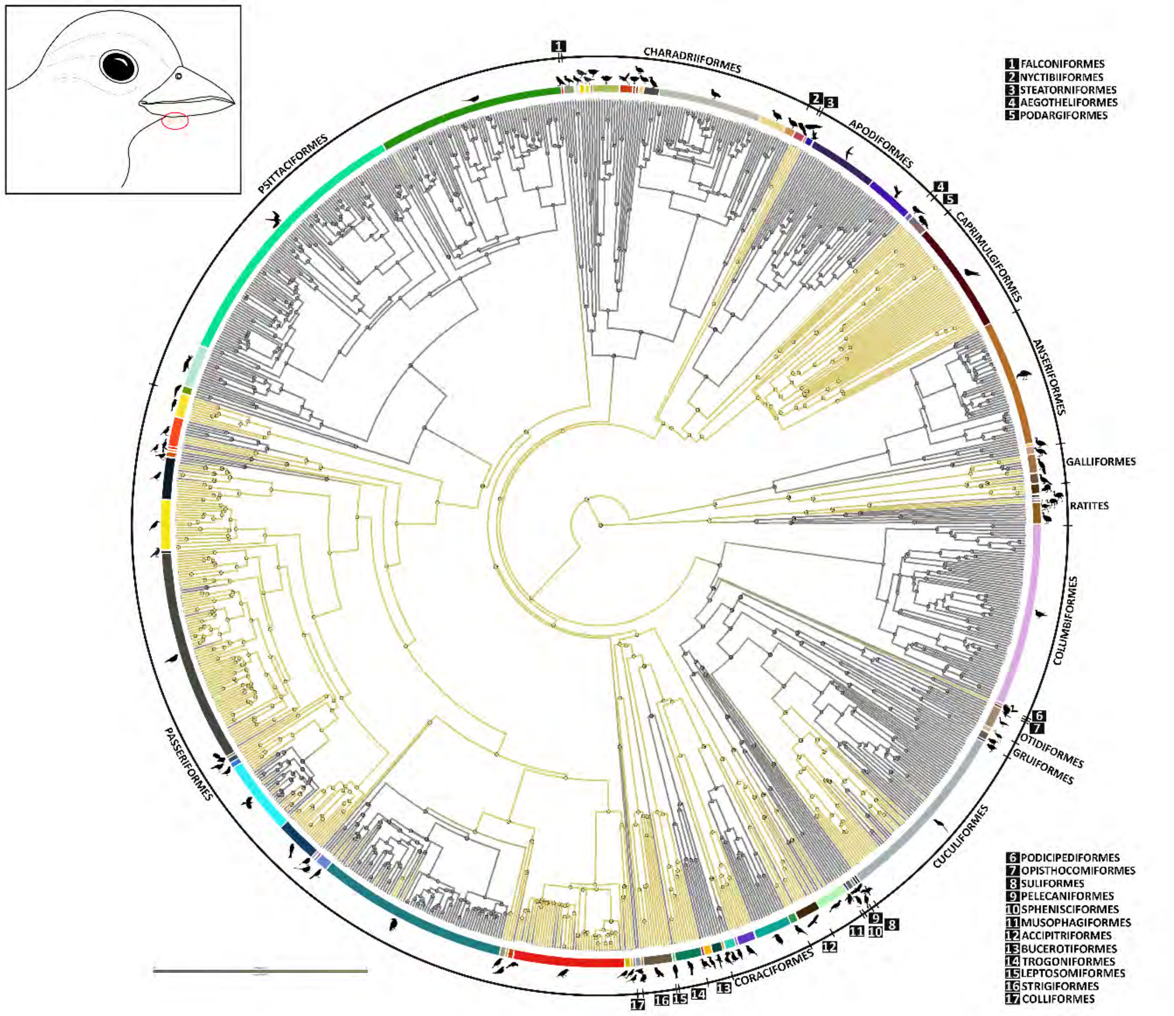


Figure 5-10. Zoomed in phylogenetic tree mapping the ancestral character estimation for the presence of interramal bristles. Terminal branches correspond to the different species measured; yellow branches indicates the high probability of the presence while grey, the absence. The inner coloured arc encircling the radial phylogenetic tree with bird silhouette illustrates the avian family each species belongs to, and the black outer circle correspond to the avian orders.

Table 5-3. **Ancestral state reconstruction** (ASR) **of the stochastic character facial bristle type for each location**: upper rictus, lores, lower rictus, nares and interramus, with their phylogenetic signal on to the phylogeny.

Facial bristle locations	Average n of changes	Gain	Loss	Phylogenetic Signal	Ancestral character state	Confidence in ASR	Time spent as absent
Upper rictus	63	16	47	λ 0.89 $p<0.001$	Present	87%	60.6%
Lores	72	55	17	λ 0.81 $p<0.001$	Absent	98.5%	77%
Lower rictus	127	3	124	λ 0.73 $p<0.001$	Present	99.9%	66.8%
Nares	74	52	22	λ 0.95 $p<0.001$	Absent	98.4%	71.61%
Interramus	87	23	64	λ 0.89 $p<0.001$	Present	96.8%	60.9%

Sensitivity analyses

Stochastic character mapping based on 100 randomly sampled trees from the posterior distribution over 100 simulations gave similar results to the stochastic character mapping with the consensus tree for rictal bristle presence and facial bristle location (Table 5-4). Stochastic character mapping based on the weighted subsampled datasets (70%, 80% and 90% of species) also reported similar numbers (Table 5-4) to those obtained by mapping based on the full dataset, and the dataset representing species with only rictal bristles present (Table 5-4); thereby confirming the validity of the consensus tree analysis.

Although, all sensitive analyses conducted for the rictal bristle shape gave similar results to the stochastic character mapping with the consensus tree, the ancestral character given with the mapping based on 100 randomly sampled trees from the posterior distribution over 100 simulations and on the 70% weighted subsampled dataset differed (Table 5-4). Both analyses gave branched instead of barbs at the base as the ancestral character, however the percentage of confidence in the ASR was lower (<75%) than the analysis based on 90% and 80% weighted subsampled datasets (>85%) (Table 5-4); therefore, with higher confidence in the ASRs, the consensus tree analysis can be validated.

Table 5-4. Sensitivity analysis results: mapping of the stochastic character on 100 trees randomly sampled from the posterior distribution over 100 simulations, and mapping of the stochastic character with each weighted downsampled subsets (70%, 80%, 90%).

Analysis	Character	Rictal bristle presence	Upper rictus	Lores	Lower rictus	Nares	Interramus
Random trees	**Average of changes**	65	65	75	127	76	94
	Gains/Losses	15/50	15/50	56/19	6/121	53/23	14/80
	Time spent as absent	62%	63%	81%	65%	75%	62%
	Ancestral character state	Present	Present	Absent	Present	Absent	Present
	Confidence in ASR	90%	90%	98%	99%	98%	99%
Subset 90%	**Average of changes**	55	55	67	119	72	82
	Gains/Losses	18/37	16/39	50/17	3/116	50/22	19/63
	Time spent as absent	61%	60%	77%	66%	71%	60%
	Ancestral character state	Present	Present	Absent	Present	Absent	Present
	Confidence in ASR	70%	87%	100%	100%	98%	98%
Subset 80%	**Average of changes**	50	57	57	105	64	73
	Gains/Losses	14/36	11/46	46/11	4/101	46/17	18/55
	Time spent as absent	61%	59	77%	67%	71%	62%
	Ancestral character state	Present	Present	Absent	Present	Absent	Present
	Confidence in ASR	85%	96%	98%	100%	98%	96%
Subset 70%	**Average of changes**	51	51	50	93	52	75
	Gains/Losses	17/34	15/36	41/9	8/85	37/15	4/71
	Time spent as absent	60%	60%	77%	66%	73%	56%
	Ancestral character state	Present	Present	Absent	Present	Absent	Present
	Confidence in ASR	79%	79%	98%	99%	97%	100%

5.3.2 Gains, losses and morphology patterns across the selected clades phylogeny

Rictal bristles appeared in Caprimulgiformae (nightjars and allies), Glareolidae (pratincoles and coursers) of the Charadriiformes (gulls, auks, jacanas, plovers, sandpipers and allies), Strigopidae (New Zealand parrots) of the Psittaciformes (parrots and cockatoos), Coliiformes (mousebirds), Strigiformes (barn-owls and owls), Trogoniformes (trogons), Coraciiformes (todies, motmots, kingfishers, bee-eaters, rollers, and ground-rollers), Accipitriformes (secretarybird, ospreys, hawks, eagles, and kites), Cuculiformes (cuckoos), Opisthocomiformes (hoatzin), a single species (houbara bustard, *Chlamydotis undulata*) from the Otidiformes (bustards) and Passeriformes (songbirds, flycatchers, oscines and allies). Within the Passeriformes, rictal bristles were present in Tyrannidae, Meliphagidae, Artamidae, Oriolidae, Platylophidae, Dicruridae, Mimidae, Turdidae, Muscicapidae, Ploceidae, Emberizidae, Corvidae, Icteridae, Motacillidae, and Sturnidae. Whilst some clades (e.g. Anseriformes and Columbiformes) were uniformly characterised by the absence of bristles across all species measured, other groups, such as the Palaeognathae, the Caprimulgiformae and the Passeriformes were selected as 'clades of interest' as characterised by multiple changes in character state.

Palaeognathae

Rictal bristles were found in Apterygidae, Rheidae and Struthionidae but were absent in Casuariidae (*Casuarius* genus) and Tinamidae (Figure 5-11). Rictal bristle presence was ancestral (80% confidence in ASR) and then lost independently on two occasions within the Palaeognathae clade (Figure 5-11). No events of return of the ancestral state appeared to have occurred. Rheidae and Struthionidae had unbranched rictal bristles with short normalised bristle lengths (0.000423 and 0.0000691, respectively; dark blue area shown in Figure 5-3A). Apterygidae, however, all had similar normalised rictal bristle lengths, which were longer than the Rheidae and the Struthionidae rictal bristles but still relatively short (between 0.011 and 0.018 across species; dark blue in Figure 5-3A). All but one kiwi species had unbranched rictal bristles, except one, *Apteryx australis*, which had rictal bristles with barbs at the base (Figure 5-3B).

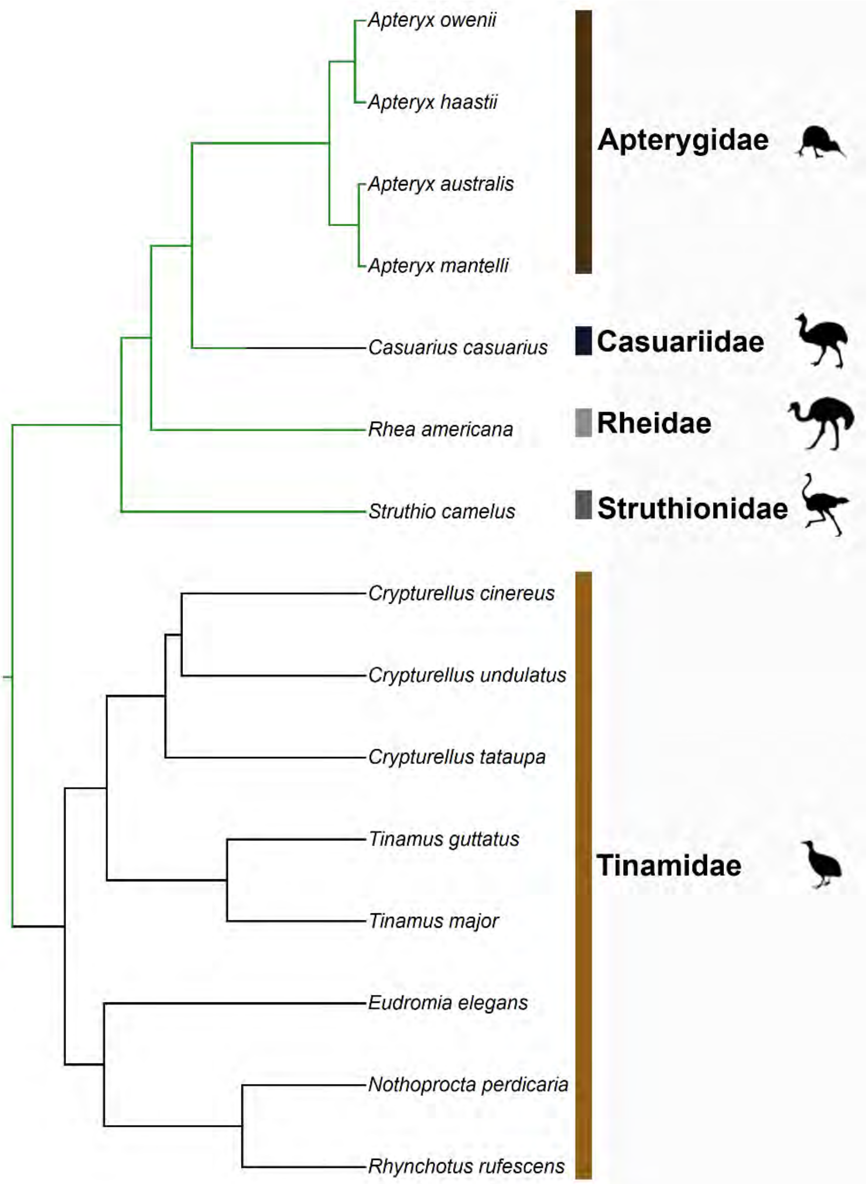


Figure 5-11. **Ratites and tinamous phylogeny clade** extracted from the avian radial phylogeny tree used for rictal bristle character presence mapping (presence shown in green, absence shown in black).

Caprimulgiformae

The Caprimulgiformae clade included six different orders of eponymous families consisting of the Caprimulgiformes, Steatornithiformes, Nyctibiiformes, Podargiformes, Aegotheliformes and Apodiformes, which included the families Hemiprocnidae, Apodidae and Trochilidae. Within the Caprimulgiformae, rictal bristles were only absent in the Apodiformes order (Figure 5-12). Rictal bristles were present in the last common ancestor of the Caprimulgiformae (100% confidence in ASR) and were then independently lost once in the last common ancestor of Apodidae, Hemiprocnidae and Trochilidae, and conserved in the other Caprimulgiformae families (Figure 5-12). No losses or gains were evident based on the species measured.

Normalised rictal bristle length and rictal bristle shape varied across the Caprimulgiformae. Both Aegothelidae species had branched rictal bristles (Figure 5-3B) with a medium normalised length (cyan in Figure 5-3A; Table 5-5). Similar to the Aegothelidae, Podargidae species all had branched rictal bristles (Figure 5-3B), however, their normalised rictal bristle lengths vary, from short to medium-short (~0.225) normalised length (Figure 5-3A; Table 5-5). Nyctibiidae species all had unbranched bristles, except for one species, *N. grandis*, which had branched rictal bristles (Figure 5-3B; Table 5-5). Nyctibiidae had short normalised rictal bristle lengths, shorter than Aegothelidae (Figure 5-3A; Table 5-5). Most Caprimulgidae species had unbranched rictal bristles (79%, n=33 species) while the rest had branched rictal bristles (n= 9) (Figure 5-3B). Their normalised rictal bristle lengths ranged from short to long (dark blue to light yellow-green in Figure 5-3A; Table 5-5). Caprimulgidae species with the shortest normalised rictal bristle length had a branched shape (Table 5-5), whereas normalised rictal bristle length longer than 0.169343 had unbranched rictal bristles. Finally, the sole Steatornithidae species, *S. caripensis* (oilbird) had relatively short normalised rictal bristle length, with unbranched rictal bristles (Figure 5-3; Table 5-5).

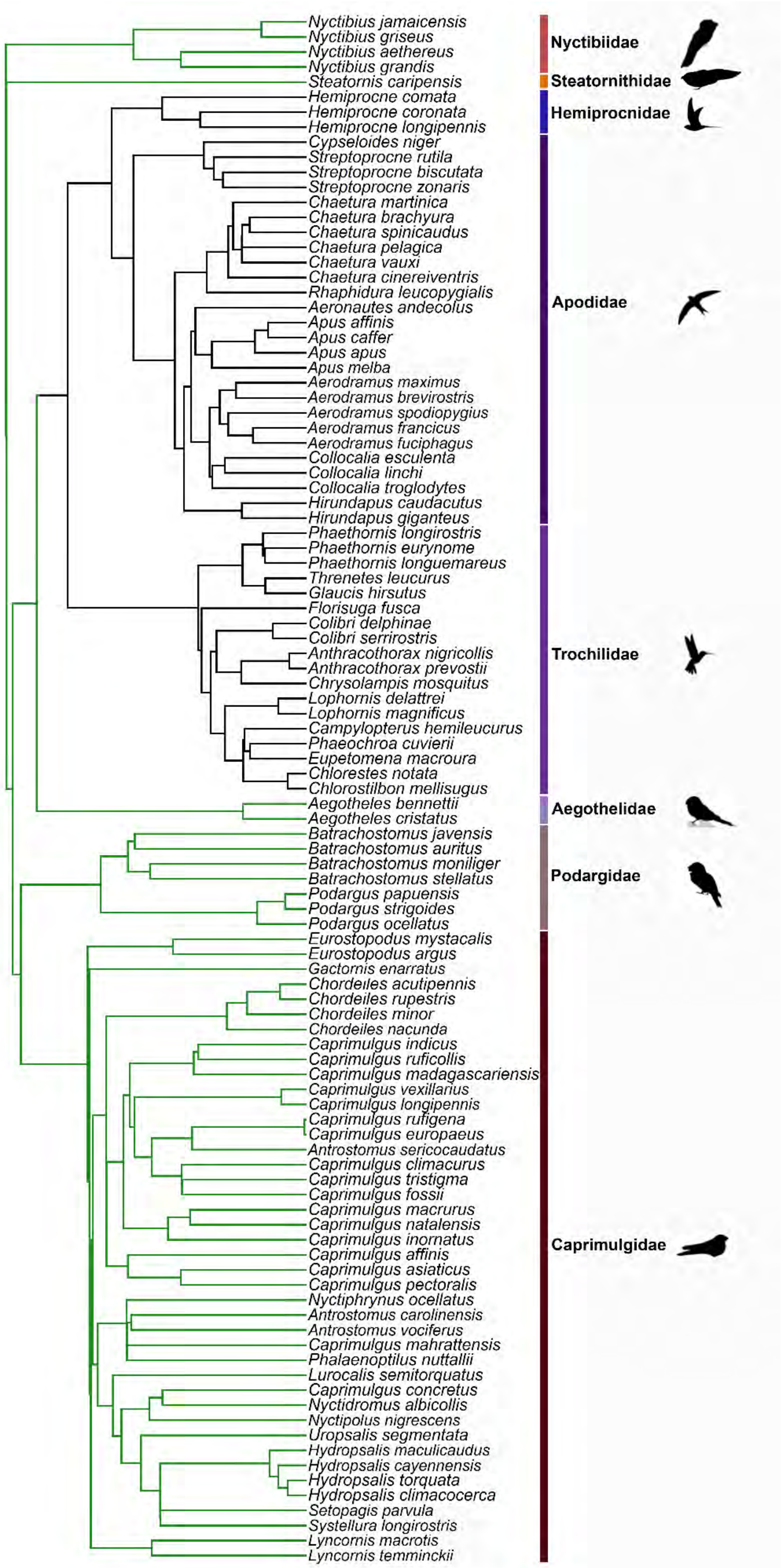


Figure 5-12. **Caprimulgiformae phylogeny clade** extracted from the avian radial phylogeny tree used for the rictal bristle presence character mapping (presence in green, absence in black). Caprimulgiformae includes the families Caprimulgidae, Podargidae, Steatornithidae, Nyctibiidae, Trochilidae, Hemiprocnidae and Apodidae.

Table 5-5. Rictal bristle measurement in the Caprimulgiformae. Rictal bristle (RB) were present in Aegothelidae, Caprimulgidae, Nyctibiidae, Podargidae, and Steatornithidae.

Family	Species	Normalised RB length	Length category	RB morphology
Aegothelidae	*Aegotheles bennetii*	0.288056	Medium	Branched
	Aegotheles cristatus	0.461275	Medium	Branched
Caprimulgidae	*Caprimulgus affinis*	0.269591	Medium	Unbranched
	Caprimulgus asiaticus	0.364484	Medium	Unbranched
	Antrostomus carolinensis	0.231269	Medium	Unbranched
	Hydropsalis cayennensis	0.59554	Medium	Unbranched
	Caprimulgus climacurus	0.330058	Medium	Unbranched
	Caprimulgus concretus	0.229525	Medium	Unbranched
	Gactornis enarratus	0.318182	Medium	Unbranched
	Caprimulgus europaeus	0.247015	Medium	Unbranched
	Caprimulgus fossii	0.245039	Medium	Unbranched
	Caprimulgus indicus	0.229374	Medium	Unbranched
	Caprimulgus inornatus	0.308065	Medium	Unbranched
	Systellura longirostris	0.607639	Medium	Unbranched
	Caprimulgus macrurus	0.292674	Medium	Unbranched
	Hydropsalis maculicaudus	0.573982	Medium	Unbranched
	Caprimulgus madagascariensis	0.378548	Medium	Unbranched
	Caprimulgus mahrattensis	0.239327	Medium	Unbranched
	Caprimulgus natalensis	0.198842	Short	Unbranched
	Nyctipolus nigrescens	0.413399	Medium	Unbranched
	Setopagis parvula	0.492342	Medium	Unbranched
	Caprimulgus pectoralis	0.410752	Medium	Unbranched
	Caprimulgus ruficollis	0.241606	Medium	Unbranched
	Caprimulgus rufigena	0.273127	Medium	Unbranched
	Antrostomus sericocaudatus	0.456928	Medium	Unbranched
	Caprimulgus tristigma	0.234072	Medium	Unbranched
	Antrostomus vociferus	0.573796	Medium	Unbranched
	Chordeiles acutipennis	0.086569	Short	Branched
	Chordeiles minor	0.050021	Short	Branched
	Chordeiles rupestris	0.057328	Short	Branched
	Eurostopodus argus	0.086376	Short	Branched
	Lyncornis macrotis	0.040885	Short	Branched
	Eurostopodus mystacalis	0.063435	Short	Branched
	Lyncornis temminckii	0.048406	Short	Branched
	Hydropsalis climacocerca	0.398188	Medium	Unbranched
	Hydropsalis torquata	0.392735	Medium	Unbranched
	Lurocalis semitorquatus	0.074989	Short	Branched
	Caprimulgus longipennis	0.267188	Medium	Unbranched
	Caprimulgus vexillarius	0.169343	Short	Unbranched
	Nyctidromus albicollis	0.364727	Medium	Unbranched
	Nyctiphrynus ocellatus	0.507576	Medium	Unbranched
	Phalaenoptilus nuttallii	0.370715	Medium	Unbranched
	Chordeiles nacunda	0.058124	Short	Branched
	Uropsalis segmentata	0.704351	Long	Unbranched
Nyctibiidae	*Nyctibius aethereus*	0.034491	Short	Unbranched
	Nyctibius grandis	0.034415	Short	Branched
	Nyctibius griseus	0.098669	Short	Unbranched
	Nyctibius jamacensis	0.064229	Short	Unbranched
Podargidae	*Batrachostomus auritus*	0.095065	Short	Branched
	Batrachostomus javensis	0.312545	Medium	Branched
	Batrachostomus moniliger	0.211635	Short	Branched
	Batrachostomus stellatus	0.361641	Medium	Branched
	Podargus ocellatus	0.111574	Short	Branched
	Podargus papuensis	0.048195	Short	Branched
	Podargus strigoides	0.075223	Short	Branched
Steatornithidae	*Steatornis caripensis*	0.095874	Short	Unbranched

Passeriformes

Rictal bristles were present in the last common ancestor of the Passeriformes order (100% confidence in ASR) with 12 independent loss events and three gains within the Order (Figure 5-2, Figure 5-13A). Rictal bristles were lost in the last common ancestor of Corcoracidae (White-winged Chough and Apostlebird), Callaeidae (Wattlebirds), and Cnemophilidae (Satinbirds) (84%, 97% and 66% confidence in ASR, respectively). They were present in the last common ancestor of Aegithalidae (Long-tailed tits) and Paridae (Tits, Chickadees, and Titmice) but lost in these two families (80% confidence in ASR). However, gains and losses appeared within the Emberizidae, Ploceidae, Sturnidae, Paradisaeidae, Corvidae, Artamidae and Ptilonorhynchidae, while their last common ancestor had rictal bristles present (97-100% confidence in ASR) (Figure 5-13B-G). Indeed, in Emberizidae, while only two species were recorded overall, one had rictal bristles while the other had not (Figure 5-13B). In Ploceidae (Weavers and allies), rictal bristles were lost for the last common ancestor of the *Malimbus* and *Ploceus* genera (100% confidence in ASR), while three *Ploceus* species secondarily gained rictal bristles (Figure 5-13B). In Sturnidae (Starlings), Paradisaeidae (Birds-of-paradise), Corvidae (Crows, Jays, and Magpies), Artamidae (Woodswallows, Bellmagpies, and allies) and Ptilonorhynchidae (Bowerbirds) rictal bristle presence was lost independently multiple times (at least 10 times) but no re-appearance of the rictal bristles occurred (Figure 5-13C-G).

Most Passeriformes species had unbranched rictal bristles (94% of the species measured with bristles, n=217 species, Figure 5-3B), however, nine Corvidae species, a single Ploceidae species (*P. nelicourvi*) and three species of Sturnidae had rictal bristles with barbs at the base (Figure 5-3B). Similarly, most Passeriformes species had short normalised rictal bristle length (87% of Passeriformes species, n=202, had a length<0.225; dark blue in Figure 5-3B). A small number of Passeriformes species had medium normalised rictal bristle lengths (7%, n=17), and were part of the Dicruridae, the Tyrannidae, the Rhipiduridae, and the Platylophidae families (light blue to cyan-green in Figure 5-3B). Finally, only one species belonging to the Dicruridae family (*Dicrurus atripennis*) had long relative normalised rictal bristle lengths (0.913882; yellow in Figure 5-13B).

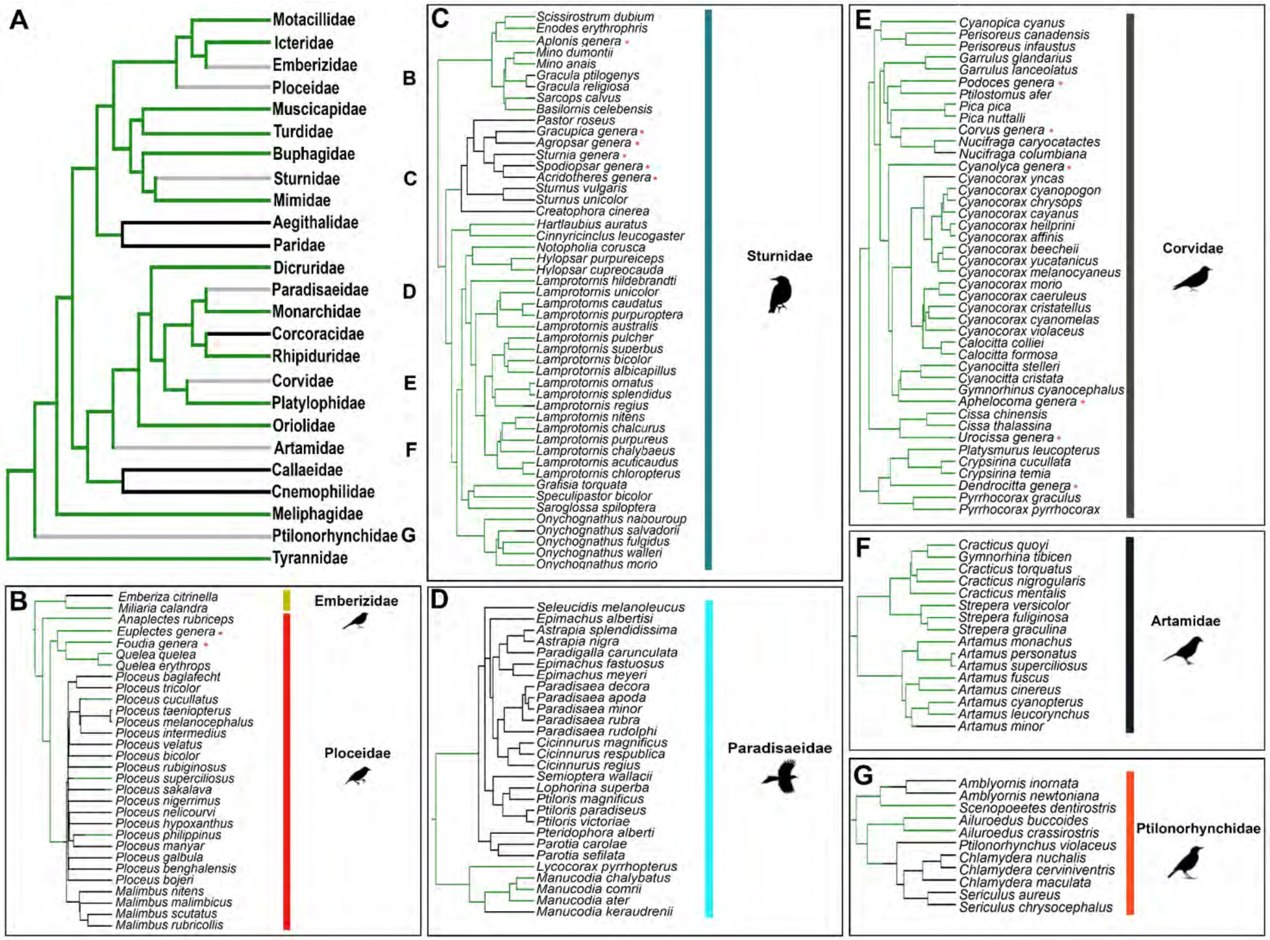


Figure 6-13. **Clades of the Passeriformes order phylogeny** extracted from the avian radial phylogeny tree used for the rictal bristle presence character mapping (presence in green, absence in black). A) Phylogeny on to which branches were collapsed to only show families; B) Clade with Emberizidae and Ploceidae; C) Sturnidae family clade; D) Paradisaeidae family clade; E) Corvidae family clade; F) Artamidae family clade; G) Ptilonorhynchidae family clade. * indicates when branches were collapsed to genera.

5.4 Discussion

Rictal bristles were likely to be present in the ancestor of modern birds, or at least at the basal node of the phylogeny used in the present analysis, possibly around 108 mya. During their evolution, the class Aves has undergone considerable morphological diversification resulting in the loss, gain or modification of rictal bristles at all levels of phylogenetic relatedness. This suggests that phylogeny alone does not explain their occurrence and form, and that adaptation to changes in ecological gradients might also be important. Ancestral rictal bristles underwent multiple occurrences of disappearances and gains, and varied in length, shape and location. Ancestral rictal bristles were likely to be short (normalised rictal bristle length <0.225), with barbs at the base. Facial bristles were also most likely present in the last common ancestor on the upper and lower rictus, and interramus, and were most likely absent on the lores and nares. Although there is only putative evidence regarding their function, they may have been mechanosensory to guide navigation or foraging, or play a role in protecting the eyes.

Evolution of avian bristles

The ancestral state reconstruction reveals that, since their appearance, rictal bristles have undergone multiple occurrences of disappearances and gain throughout avian evolution, especially in the order Passeriformes, which have the highest number of families within its order. Rictal bristle presence was found in the last common ancestor of ratites, Caprimulgiformae, Psittaciformes, and Passeriformes, as well as the last common ancestor of Coliiformes, Strigiformes, Leptosomiformes, Trogoniformes, Bucerotiformes and Accipitriformes (Figure 5-2). In total, this study identified 35% of all species measured had rictal bristles present, covering 39.4 % of all the evolutionary period in this study.

All avian feathers are diverse (Stettenheim, 2000; Yu et al., 2004) and this study suggests that rictal bristles are no exception, exhibiting pronounced variation in length, shape, and position across species, even within the same family. An unbranched rictal bristle shape was the most common form of rictal bristle, found in 80% of all species studied with bristles, followed by the branched shape (barbs and barbules covering most of the bristle length), which was found in 13% of the species studied with bristles. Despite the prevalence of unbranched bristles throughout the phylogeny, and the previous commonly held belief that the first stage of early feather evolution were unbranched bristles (Prum and Brush, 2002), this study suggests that this was not the likeliest ancestral state of rictal bristles, but rather, it was a bristle shape with barbs at the base.

Despite having a strong phylogenetic signal within the full dataset, multiple events of disappearance and gains of rictal bristles occurred within the same clades, and even within the same genus (e.g. Passeriformes, Figure 5-13). Variation in rictal bristle morphology was also observed within the same order, families and genera (e.g. Caprimulgiformae and Passeriformes, Figure 5-3, 4). Therefore, phylogeny does not seem to be the only driver influencing rictal bristles presence and morphology (length and shape). There may be common evolutionary events and life-history traits that can account for the diversity of rictal bristle morphology (length and shape) observed across the phylogeny. Indeed, competition for niches has enabled birds to exploit diverse resources (Remsen and Robinson, 1990; Felice et al., 2019; Rico-Guevara et al., 2019), which have resulted in differences in life-history traits across species (Roots, 2006). For instance, nocturnal, raptorial and pelagic ecology have arisen multiple times in many different avian species (Hackett *et al.*, 2008). The presence/absence and variation of rictal bristle morphology could be associated with such traits. As previously shown in Chapters 3, species within the Caprimulgiformae, which tend to forage pre-dusk in open-habitats, have shorter branched rictal bristles and lack mechanoreceptors at their follicles, while similar nightjar species that prefer foraging in more scotopic conditions had mechanoreceptors. Cunningham *et al.*, (2011) found that brown kiwis (*Apteryx mantelli*), morepork (*Ninox novaeseelandiae*), stitchbird (*Notiomystis cincta*), South Island robin (*Petroica australis*) and New Zealand fantail (*Rhipidura fuliginosa*) all had functional tactile rictal bristles to some degree since mechanoreceptors were present around the follicles. They also suggested that in these species, rictal bristles may also play a role in protecting the eyes since the aforementioned New-Zealand species are all insectivorous. While kiwis and morepork are nocturnal, the stitchbird, robin and fantail are diurnal (Cunningham et al., 2011; Billerman et al., 2020).

On the other hand, the multiple evolutionary events in rictal bristles presence and the variation in morphology could suggest that rictal bristles are a plastic trait (DeWitt et al., 1998) and its form or presence may be constrained by the cost of function (Pigliucci, 2001) or the phenotype. While phenotype costs are genotype-specific and environmentally dependent, plasticity costs are genotype specific that are not dependent on the environment since plastic genotypes exist in all environments (Murren et al., 2015).

Therefore, if rictal bristles or certain bristle forms are not advantageous to a species, they could be lost or reduced to a less costly phenotype or plastic form. For instance, the disappearance of rictal bristles in Apodidae and their conservation in the other Caprimulgiformae could suggest that in Apodidae, the cost of maintenance of the rictal bristle was higher than the cost of reducing the trait. Therefore, perhaps the different morphologies are limited by their cost of maintenance and functional advantage, such as for secondary sexual characters (Møller, 1996). Rictal bristles have also appeared or re-appeared in species, which could suggest the functional gain of having bristles might have offset their production cost, which measures the cost saved by not producing the character in a non-inducing environment, and maintenance cost (DeWitt et al., 1998).

Cunningham *et al.* (2011) suggested that facial bristles might play roles in prey detection and handling, gathering of information whilst foraging on the wing and navigating in dark environments (e.g. nest cavities). Bristles covering the nostrils (narial bristles) were likely absent in the MRCA, however they evolved towards gain throughout the time covered by the tree in this study, which could suggest their presence is advantageous to birds, i.e. they may represent “hygienic structures”, shielding the nostrils from dust and other particles (Conover and Miller, 1980; Cunningham et al., 2011). Interramal and lower rictal bristles, however, were likely to have been present in the MRCA and evolved towards loss, therefore, they may not play a sufficient role in certain species to be conserved. Nonetheless, in morepork and kiwis, the follicles of interramal bristles are well-innervated and are surrounded by Herbst corpuscles, suggesting a potential tactile function, perhaps playing a role in prey manipulation or in locating offspring in cavity nests (Cunningham et al., 2011). Variation in lower rictal bristle presence across species tend to suggest that they may be more advantageous in species with narrower beaks (Passeriformes, Strigopidae, Strigiformes, Trogoniformes, Momotidae and Numididae, Coraciidae, Cuculidae, Cracidae; Billerman et al., (2020)) than larger beaks, i.e. they are absent in most Caprimulgiformae species. However, lower rictal bristles are present in Podargidae and Nyctibiidae, which are the biggest species of the order and have the largest beaks (Billerman et al., 2020). Lower rictal bristles may act as a net, catching food to throw to the back of the throat. This may be redundant in Caprimulgiformae that possess long rictal bristles that are likely tactile (Cunningham et al., 2011; Delaunay et al., 2020). In Podargidae and Nyctibiidae, perhaps having lower rictal bristles while having wide beaks might have a functional advantage, or the cost of reducing the trait might not be advantageous. Contrarily, lorial bristles were likely absent in the MRCA, and found in species that possess rictal bristles, which could suggest that lorial bristles might have evolved to expand the surface area covered by the rictal bristles, and therefore perhaps to improve protection or detection further.

Are rictal bristle different morphologies homologous structure?

It is also worth bearing in mind that the presence of many events of bristle disappearance and, especially re-apparitions, in addition to their diverse morphologies, may suggest that rictal bristles might not be a homologous structure. Some authors have suggested that 'filamentous feathers' might be similar to the bristles of the wild turkey (*Meleagris gallopavo*), which are epidermal outgrowths, rather than feathers (e.g. Sawyer *et al.*, 2003). These outgrowths leave corneous caps and hollow bristles, with no associated follicles (Lucas and Stettenheim, 1972; Sawyer et al., 2003). If this was the case, then branched bristles or semi-bristles would not be homologous with unbranched bristles. However, in Chapter 3, both unbranched and branched rictal bristles in Caprimulgiformae possessed clear follicles, some of which were also associated with mechanoreceptors. Cunningham *et al.*, (2011) also demonstrated that rictal bristles of kiwis (*Apteryx mantelli*) had follicles and were of a "feather-type". Therefore, both branched and unbranched rictal bristles, at least in Caprimulgiformae and Apterygidae, are a type of feather, rather than a bearded feather or an epidermal outgrowth.

In addition, research has suggested that "filamentous feathers" found in coelurosaurs were homologous to "true feathers" or pennaceous feathers of modern birds, and that they probably preceded them phylogenetically (Gauthier *et al.*, 2001; Padian, 2001). Similarly, branched and unbranched bristle morphologies are likely to be homologous but future work needs to verify if genetic pathways are conserved between different rictal bristle morphologies, and following gain events, in order to truly confirm homology. Anatomical studies are also needed to compare rictal bristle follicle anatomy between more species since the feather morphology is not necessarily associated with a tactile function (e.g. Chapter 3).

Conclusion

This study revealed that rictal bristles, as well as interramal and lower rictal bristles, were present in the MRCA of the avian phylogeny used here, while narial and lorial were absent. It also revealed that rictal bristle morphology was most likely short bristles with barbs at the base in the MRCA. Rictal bristle evolution underwent multiple events of gains and losses, suggesting that phylogenetic relatedness is not the only driver of rictal bristle presence and morphology. Further work should explore the genetic basis of rictal bristles, in order to verify if different rictal bristle morphologies are homologous. Future work should also explore the association of rictal bristle morphology with ecological traits in order to make predictions about bristle function.

Chapter 6

Linking ecological traits with rictal bristle function

Chapter summary:

Little is known about rictal bristle function, although bristles have been suggested to help in foraging, protecting the eyes and probably have a tactile function. This study investigated the association of foraging and habitat traits with the presence of rictal bristles and normalised rictal bristle length in 1,022 avian species - including 91 families and 29 orders. Caprimulgiformae and the Passeriformes order were also separately investigated. Results revealed that rictal bristle presence and normalised length were associated with species-specific ecological traits, after accounting for phylogenetic relatedness. Nocturnality and some aspects of foraging, such as foraging method, are likely to contribute to species having long, present rictal bristles. Diet is also associated with normalised rictal bristle length in some groups. Specifically, in Caprimulgiformes, rictal bristles are likely to be present in species foraging in low-light conditions and longer in species with unbranched rictal bristles. Whereas in Passeriformes, rictal bristles are likely to be longer in species that glean-sally and in frugivores or invertivores. This study supports my predictions from previous chapters and demonstrates that species foraging in low-light conditions are likely to have prominent, tactile rictal bristles, while species foraging in lighter conditions and during daylight might have a reduced tactile function. Additionally, rictal bristles may be absent in species that travel at high-speeds in air or water, to prevent damage and reduce drag. Therefore, birds foraging in complex habitats in the dark are most likely to have long rictal bristles that may play a role in eye protection and collision avoidance.

6.1 Introduction

Facial bristles, and especially rictal bristles, are the least described feather type and their function remains relatively unknown. It has been suggested that bristles function as a funnel or net by increasing the effective size of the gape to aid prey capture, or for protecting the eyes from vegetation and food parts while foraging (Keast and Saunders, 1991; Cunningham et al., 2011). Rictal bristles are also thought to carry out a tactile function (Lederer, 1972; Keast and Saunders, 1991; Cunningham et al., 2011; Delaunay et al., 2020). They also protect the eyes, which has been positively identified from observations and experiments with aerial insectivorous birds (Conover and Miller, 1980; Cunningham *et al.*, 2011).

The study in Chapter 5 demonstrated that rictal bristle presence, length and shape varied across the avian phylogeny, but that these variations could not simply be predicted by phylogenetic relationships alone. It is likely that ecological factors are also associated with bristle presence and morphology. Indeed, I suggested in Chapter 3 that rictal bristle follicle anatomy varies between different Caprimulgiform species, and might play a tactile role in nocturnal species that forage in closed habitats. Specifically, tactile mechanoreceptors in the immediate surroundings of the bristle follicle are absent in high aerial foraging Caprimulgiform species that prefer open habitats, species that also have short, thin, branching rictal bristles (Delaunay *et al.*, 2020; Chapter 3). Furthermore, rictal bristles are absent in diurnal Caprimulgiformes (i.e. Apodidae, Hemiprocnidae, Trochilidae families), but are present in species that forage in low light conditions (nocturnality spectrum, i.e. including nocturnal-cathemeral to obligate nocturnal species). Therefore, in Caprimulgiformes, species with long, tactile bristles may be nocturnal or crepuscular. However, not all genera within families, and not all members of each species, possess bristles. Bristles can also be absent from many species with similar life-histories to those species that possess them (Chapter 5). Therefore, comparing the presence and morphology of rictal bristles across species with multiple, potentially influencing, ecological traits is required to determine the function of these specialised feathers in the bird families in which they occur, and need to have a well-supported phylogenetically-controlled methodology.

Here, I investigate whether rictal bristle presence and length are associated with their shape and with a range of ecological traits that are known to influence other avian traits, such as beak shape and visual capabilities, such as light photosensitivity (i.e. diurnal vs nocturnal visual features). These include the period of activity (diurnal, diurnal cathemeral, nocturnal cathemeral, crepuscular, nocturnal), habitat density (open, semi-open, closed), foraging method (e.g. sallying, hawking, gleaning, diving, dabbling, probing), foraging height (low, various, high), and diet (e.g. invertivore, vertivore, frugivore, granivore).

This study will address the following hypothesis outlined in Chapter 2:

- **Variation in rictal bristle morphology will be associated with specific ecological traits**

Rictal bristle shape and anatomy are expected to be associated with ecological traits, such as nocturnality; such that nocturnal species may have longer, thicker bristles and a higher number of mechanoreceptors within their follicles.

- **Rictal bristle form and function will be associated with foraging**

Feathers evolved in different forms, shapes, sizes and textures throughout evolution. Therefore, the expected variation in rictal bristle morphology amongst species may be associated with ecological traits and so vary in function according to the bird’s ecology. More prominent rictal bristles would be expected to have a tactile function, especially in species that forage in dark, complex environments.

These results will extend my findings from chapter 3, that rictal bristle are tactile in nocturnal foragers and that a reduced tactile function might be implicated in more diurnal species that forage open-habitat (Chapter 3; Delaunay et al., 2020)

A phylogenetic comparison method will be conducted to test the association of rictal bristle presence and normalised length with ecological traits across 1,022 avian species (~10% recorded species), representing 418 genera, from 91 families (37% recorded families) and 29 orders (73% of all orders). These associations will also be further investigated in Caprimulgiformae and Passeriformes orders, which have varied rictal bristle presence and morphology across species (Chapter 5). Moreover, Caprimulgiformae are mainly on the nocturnal spectrum while Passeriformes have numerous diurnal species with rictal bristles, which makes them ideal to compare. I will model rictal bristle presence and normalised length as a function of life-history strategies by means of a linear mixed model using Bayesian approximation (MCMCglmm) as it enables the inclusion of a phylogeny as a design matrix in the model framework (Hadfield and Nakagawa, 2010). MCMCglmm allows me to investigate the association between innovative propensity and life-history traits whilst controlling for phylogenetic dependencies (Sol et al., 2016).

6.2 Material and methods

6.2.1 Data compilation: rictal bristles and ecological traits

Details of the rictal bristle dataset (presence and morphology) of 1,022 avian species and the consensus phylogenetic tree are presented in Chapter 5. Rictal bristle morphology similarly comprised of the normalised rictal bristle length and the rictal bristle shape. However for this investigation, the normalised rictal bristle length was calculated per individual (the average length of the rictal bristles measured per individual divided by the body mass of the species), rather than per species to incorporate all the individual specimens measured into the analyses.

Species-specific ecological traits were added to the dataset using birdsoftheworld.org (Billerman *et al.*, 2020), and included the following trait variables: i) period of activity, ii) habitat density, iii) foraging method, iv) foraging height, and v) diet (Table 6-1). Instances where some species had more than one foraging trait, the two main categories were combined, e.g. crepuscular and nocturnal, and gleaning and hawking (Table 6-1). Similarly, in instances where species belonged to more than one diet category, these were also grouped (with a maximum of two categories), the first group corresponding to the main food type, followed by the secondary food type, e.g. invertivore and granivore (Table 6-1).

Table 6-1. Ecological traits, categories, definition and possible combinations used in this study (diet in bold are the main diet taken by the bird).

Ecological traits	Categories	Definitions	Combinations
ACTIVITY PERIODS	**Diurnal**	Forage during the day	Crepuscular- Nocturnal
	Diurnal cathemeral	Predominantly diurnal species that occasionally foraged at dawn, dusk or even during the night	
	Nocturnal cathemeral	Predominantly nocturnal species that occasionally feed during daytime from late morning to late afternoon	
	Crepuscular	Forage at dusk and dawn and full moonlight night	
	Nocturnal	Forage during the night, in low light condition	
HABITAT DENSITIES	**Open**	Scarcely wooded or bare area (e.g. grasslands, heathlands, clearings, wetlands, marshlands, scrublands, savannahs, desert, arid, semi-arid)	None
	Semi-open	Loosely wooded area that is a mixture of open country and woodlands (e.g. corridors, woodlands)	
	Closed	High, densely wooded areas (e.g. rainforests, woodlands, evergreen forests)	
DIET	**Invertivores**	Eat flying insects or/and terrestrial invertebrates	**Invertivore**- Vertivore / Aquatic invertivore /Aquatic vertivore / Frugivore / Granivore / Nectarivore **Vertivore-** Invertivore **Aquatic Herbivore-** Aquatic invertivore / Granivore **Aquatic invertivore-** Aquatic herbivore / Aquatic vertivore / Invertivore **Aquatic vertivore-** Aquatic invertivore **Frugivore-** Granivore / Herbivore /Invertivore / Nectarivore **Granivore-** Frugivore / Herbivore / Invertivore **Nectarivore-** Herbivore / Invertivore
	Vertivores	Eat terrestrial vertebrates	
	Aquatic invertivore	Eat aquatic invertebrates, such as crustaceans and water insects	
	Aquatic vertivore	Eat aquatic vertebrates, such as fishes	
	Aquatic herbivore	Eat aquatic plants, such as seaweed and algae	
	Frugivores	Eat fruits	
	Granivores	Eat plant seeds	
	Nectarivores	Eat plant nectar and plant exudates	
	Herbivores	Eat vegetation parts, such as leaves, buds and flowers	
	Omnivore	Eat invertivore, vertivore and plants material and exudates	

FORAGING METHOD	**Skimming**	Birds feed along the surface of the water to capture prey	Gleaning- Hawking/ Sallying Hawking-Sallying Sallying- Plunge diving
	Dabbling	Birds immerse head, neck and upper body while swimming to get submerged vegetation	
	Dipping	Birds briefly submerge themselves, partially or completely, to catch food	
	Diving	Birds submerge themselves completely under water and swim to forage on vegetation or pursue prey (e.g. fishes, crustaceans)	
	Gleaning	Birds forage by pecking/picking meticulously food from surfaces such as tree bark, branch, leaves or grass and ground.	
	Hawking	Birds snatch food on the wing, consuming the prey without perching	
	Sallying	Birds sit on a perch before and after catching prey in the air	
	Lunging	Birds dart rapidly on prey and often pauses between hunting strikes.	
	Scratching	Birds loosen or remove dirt/debris from the ground with its foot or both feet to reveal food (seeds, bugs)	
	Plunge diving	Birds plunge into the water from a significant height to catch prey under the surface	
	Probing	Birds insert their beak into a crevices/gap to extract food from it	
	Scanning	Birds carefully watch, perched, hovering or soaring, over an area before launching their attack to the ground or in the water	
FORAGING HEIGHT	**Low**	Forage on the ground and in the low the canopy	None
	Various	Forage on the ground and all levels of the canopy	
	High	Forage high and above the canopy	

6.2.2 Model construction and analyses

To determine the relationship between rictal bristle presence and morphology and species-specific ecological traits, a phylogenetically controlled Markov chain Monte Carlo generalised linear mixed model (MCMCglmm) was conducted, using the R package "MCMCglmm" (Hadfield, 2010) in RStudio (R Core team, 2018). The MCMCglmm applies Bayesian statistics to fit general linear models. It included the phylogenetic variance and covariance matrix as a random effect in the regression model, to account for phylogenetic relatedness and intraspecific variation in the dataset. Accounting for phylogenetic dependencies was needed in the models here, since Chapter 5 revealed that rictal bristle presence and normalised rictal bristle length had a strong phylogenetic signal using the phylogeny used here (lambda=0.89 and 1, respectively). Models for both rictal bristle presence/absence and normalised rictal bristle length were constructed for: i) all species (full dataset); ii) the Caprimulgiformae data subset, and iii) the Passeriformes order data subset. Caprimulgiformae and Passeriformes were chosen as focus groups since the majority of species of each group possess rictal bristles, as well as variation in rictal bristle morphology (Chapter 6). Furthermore, Passeriformes species are predominantly diurnal while five out of eight families of Caprimulgiformae are nocturnal (i.e. nocturnal cathemeral, crepuscular, crepuscular-nocturnal, and nocturnal). For rictal bristle presence, a binomial 'threshold' model was used to account for the binary response variable, whereas a "gaussian" model was used for the normalised rictal bristle length continuous response variable. In both models, the period of activity, the habitat density, the foraging method, the foraging height and diet were included as fixed effects, with shape also added to the normalised rictal bristle length model as an additional fixed effect. In both models, phylogeny and individual ID were included as independent random effects.

The model was run for 800,000 iterations, with a burn-in period of 80,000, and a thinning of 40, which were determined using graphical diagnostics and by testing for autocorrelation between samples (using coda package; Plummer *et al.*, 2006). Three independent MCMC chains were run per model to check for model convergence using Gelman-Rubin diagnostics, with model convergence confirmed when the potential scale reduction factor required value was <1.1 (Gelman and Rubin, 1992). Effective sample sizes (>200) and autocorrelation ($p<0.05$) values between successive iterations were also examined. Non-significant fixed effects (*p*MCMC > 0.10) were permanently excluded from the model formula if, in doing so, the fit of the model improved. Assessment of the relative importance of predictors was conducted by comparing the Deviance Information Criterion (DIC, Spiegelhalter *et al.*, 2002) of the full model and the model with the excluded predictor (Hadfield, 2010). If the DIC was lowered by at least 2, which indicates a higher model support, the model with the excluded predictor was kept. Subsequently, diet was removed for the rictal bristle presence model in the analyses for all species, both habitat and foraging height were removed in the normalised length model, and foraging method was removed from the model for rictal bristle presence for the Passeriformes. Following (Hadfield, 2010), a weak informative inverse-Gamma prior was used, with variance (V) set to 1, and the belief parameter (nu) set to 0.002 for both the random effects structure (G-structure) and residual structure (R-structure). Residual variance was fixed in the absence of this information for the rictal bristle presence model since this used binary data (as per Hadfield, 2010). Other parameter combinations were systematically explored but the models did not converge with them. Using ggplot2 package (Wickham, 2016b), caterpillar plots representing the mean parameter estimates and the 95% credible intervals (CI) for each model were constructed and examined, i.e. if the credible intervals were found to exclude zero, the parameter was considered significant with the model *p*-value given by the *p*MCMC value.

It was not possible to obtain a converging model for bristle shape since this was a categorical variable that exhibited a large range in the number of species in each category i.e. unbranched bristle shape was found in 292 species, while the branched shape was only found in 47 species, and branched at the base in 26 species. While visual inspection of MCMC chains suggested convergence after 12.8 million iterations, convergence was not supported by the Gelman-Rubin statistic; therefore, a model for bristle shape was not considered further. In order to examine the association of bristle shape with length (as suggested in Chapter 3), the categorical shape variable was added to the fixed effects of the normalised rictal bristle length model.

Reference categories

Following model construction and validation, a suitable '*reference category'* was selected for pairwise comparisons, which are presented in Table 6-2. These are reference categories to which all others are compared, and significant differences tested for. In the full dataset, all Anatidae species were selected as reference categories for the rictal bristle presence model since rictal bristles were absent in all species. For the normalised rictal bristle length analysis, the blue-napped mousebird (*Urocolius macrourus*) was selected as the reference category for the pairwise comparisons as this species had the smallest normalised rictal bristle length measured. For the rictal bristle presence model in the Caprimulgiformae subset, the reference categories were selected based on the ecology of the Trochilidae family, which all lack rictal bristles. For the normalised rictal bristle length model in the Caprimulgiformae subset, the ecology and bristle shape of the great eared-nightjar (*Lyncornis macrotis*) with the smallest normalised rictal bristle length was used as the reference categories. For the rictal bristle presence model in the Passeriformes subset, the ecology of the Rüppel's weaver (*Ploceus galbula*) was selected as reference categories as only species without rictal bristles across the different model predictors. For the normalised rictal bristle length model in the Passeriformes subset, the ecology of the curl-crested manucode (*Manucodia comrii*) was selected as the reference categories as this species had the smallest rictal bristles within the Passeriformes dataset.

Table 6-2. Control categories selected for pairwise comparisons of the fixed effects in each model (rictal bristle presence and normalised rictal bristle length), for each dataset (all species, Caprimulgiformae and Passeriformes).

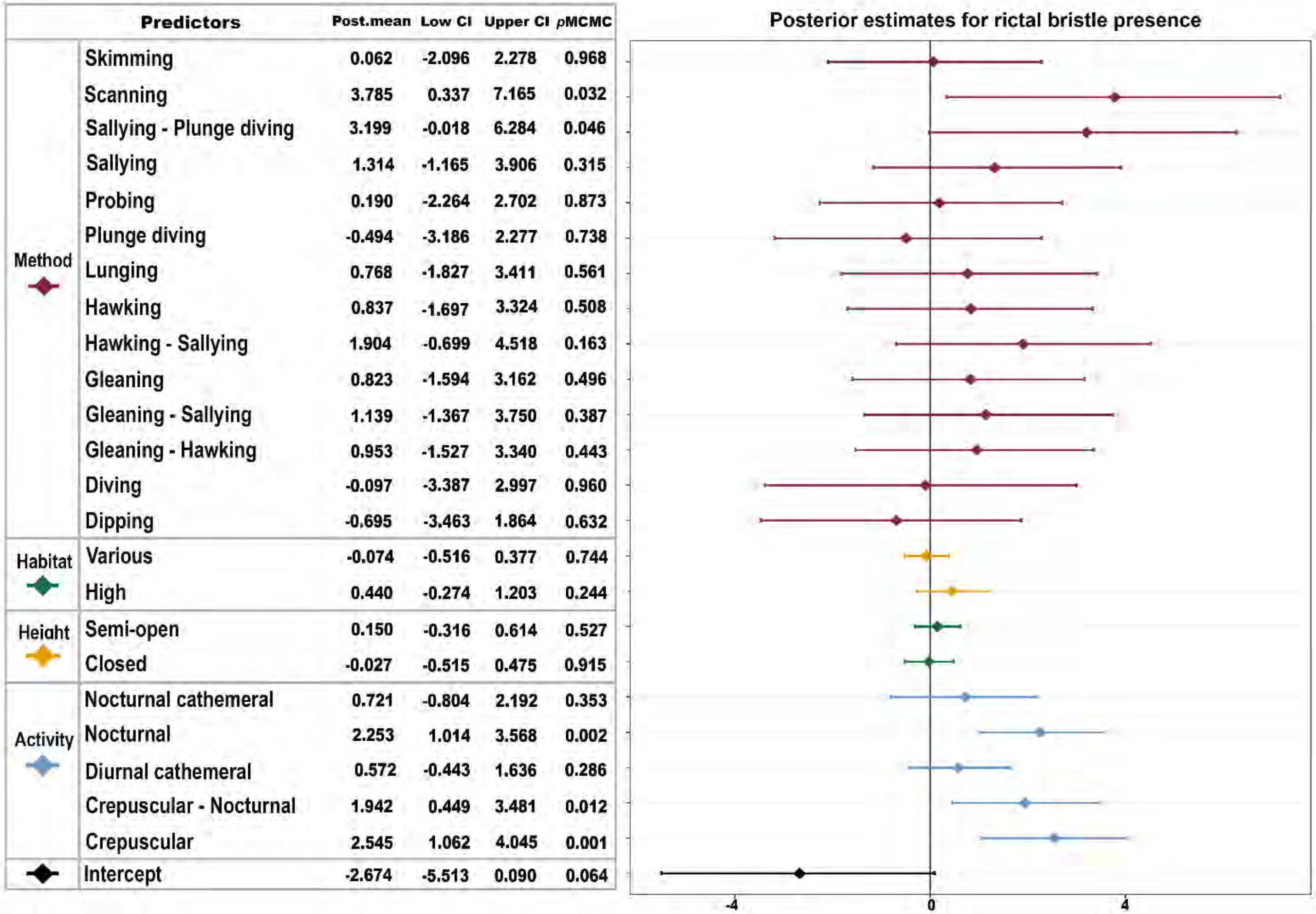
Models	Fixed effects	Activity period	Habitat density	Foraging method	Foraging height	Diet	Shape
All species	**Presence**	Diurnal	Open	Dabbling	Low	Aquatic herbivore - Granivore	Not applicable
	Normalised length	Diurnal	Open	Gleaning	Various	Frugivore - Herbivore	Unbranched
Caprimulgiformae	**Presence**	Diurnal	Open	Hawking	High	Nectarivore - Invertivore	Not applicable
	Normalised length	Crepuscular - Nocturnal	Open	Hawking	High	Invertivore	Branched
Passeriformes	**Presence**	Diurnal	Open	Gleaning	Low	Granivore	Not applicable
	Normalised length	Diurnal	Semi-open	Gleaning	Various	Frugivore - Invertivore	Unbranched

6.3 Results

6.3.1 All species

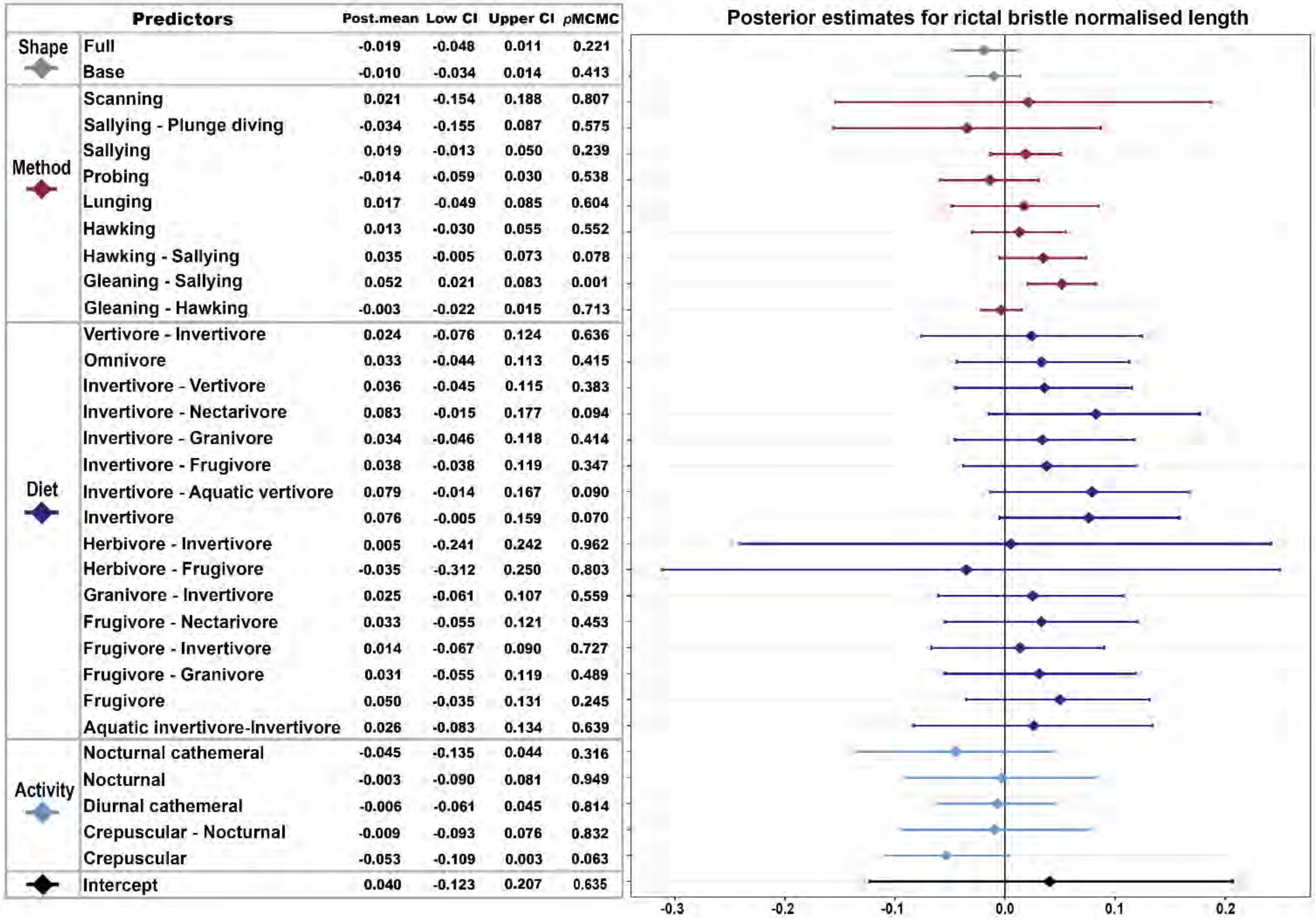
Period of activity and foraging method both significantly predicted the presence of rictal bristles. Specifically, crepuscular (*p*MCMC=0.001), crepuscular-nocturnal species (*p*MCMC = 0.012) and nocturnal species (*p*MCMC = 0.002) were more likely to have rictal bristles than diurnal species. In addition, species that foraged by sallying-plunge diving (*p*MCMC = 0.046) and scanning (*p*MCMC = 0.046) were more likely to have rictal bristles than dabbling species (Figure 6-1). By contrast, the variables diet, foraging height and habitat density did not significantly predict rictal bristle presence (Figure 6-1). For the rictal bristle presence model in all species, diet was determined as non-significant fixed effect (6.2.2).

Foraging method also significantly predicted with the normalised rictal bristle length, specifically rictal bristles were longer in species that glean and sally (*p*MCMC=0.001; Figure 6-2) compared to species that only glean. Other predictors of foraging methods, period of activity, foraging height, habitat density, diet and bristle shape did not significantly predict normalised rictal bristle length within this model (Figure 6-2). For the normalised rictal bristle length model in all species, foraging height and habitat were determined as non-significant fixed effect (6.2.2).



	Predictors	Post.mean	Low CI	Upper CI	*p*MCMC
Method ◆	Skimming	0.062	-2.096	2.278	0.968
	Scanning	3.785	0.337	7.165	0.032
	Sallying - Plunge diving	3.199	-0.018	6.284	0.046
	Sallying	1.314	-1.165	3.906	0.315
	Probing	0.190	-2.264	2.702	0.873
	Plunge diving	-0.494	-3.186	2.277	0.738
	Lunging	0.768	-1.827	3.411	0.561
	Hawking	0.837	-1.697	3.324	0.508
	Hawking - Sallying	1.904	-0.699	4.518	0.163
	Gleaning	0.823	-1.594	3.162	0.496
	Gleaning - Sallying	1.139	-1.367	3.750	0.387
	Gleaning - Hawking	0.953	-1.527	3.340	0.443
	Diving	-0.097	-3.387	2.997	0.960
	Dipping	-0.695	-3.463	1.864	0.632
Habitat ◆	Various	-0.074	-0.516	0.377	0.744
	High	0.440	-0.274	1.203	0.244
Height ◆	Semi-open	0.150	-0.316	0.614	0.527
	Closed	-0.027	-0.515	0.475	0.915
Activity ◆	Nocturnal cathemeral	0.721	-0.804	2.192	0.353
	Nocturnal	2.253	1.014	3.568	0.002
	Diurnal cathemeral	0.572	-0.443	1.636	0.286
	Crepuscular - Nocturnal	1.942	0.449	3.481	0.012
	Crepuscular	2.545	1.062	4.045	0.001
◆	Intercept	-2.674	-5.513	0.090	0.064

Figure 6-1. Caterpillar plot representing the posterior mean (post. mean) **estimates and the 95% credible intervals** (CI) **for each ecological predictor in the rictal bristle presence model in all species**. The model intercept is in black, the activity period is in blue, the habitat density is in green, the foraging height is in yellow, and the foraging method is in dark magenta, of which the reference categories were diurnal, open, dabbling low, and aquatic herbivore-granivore, respectively.

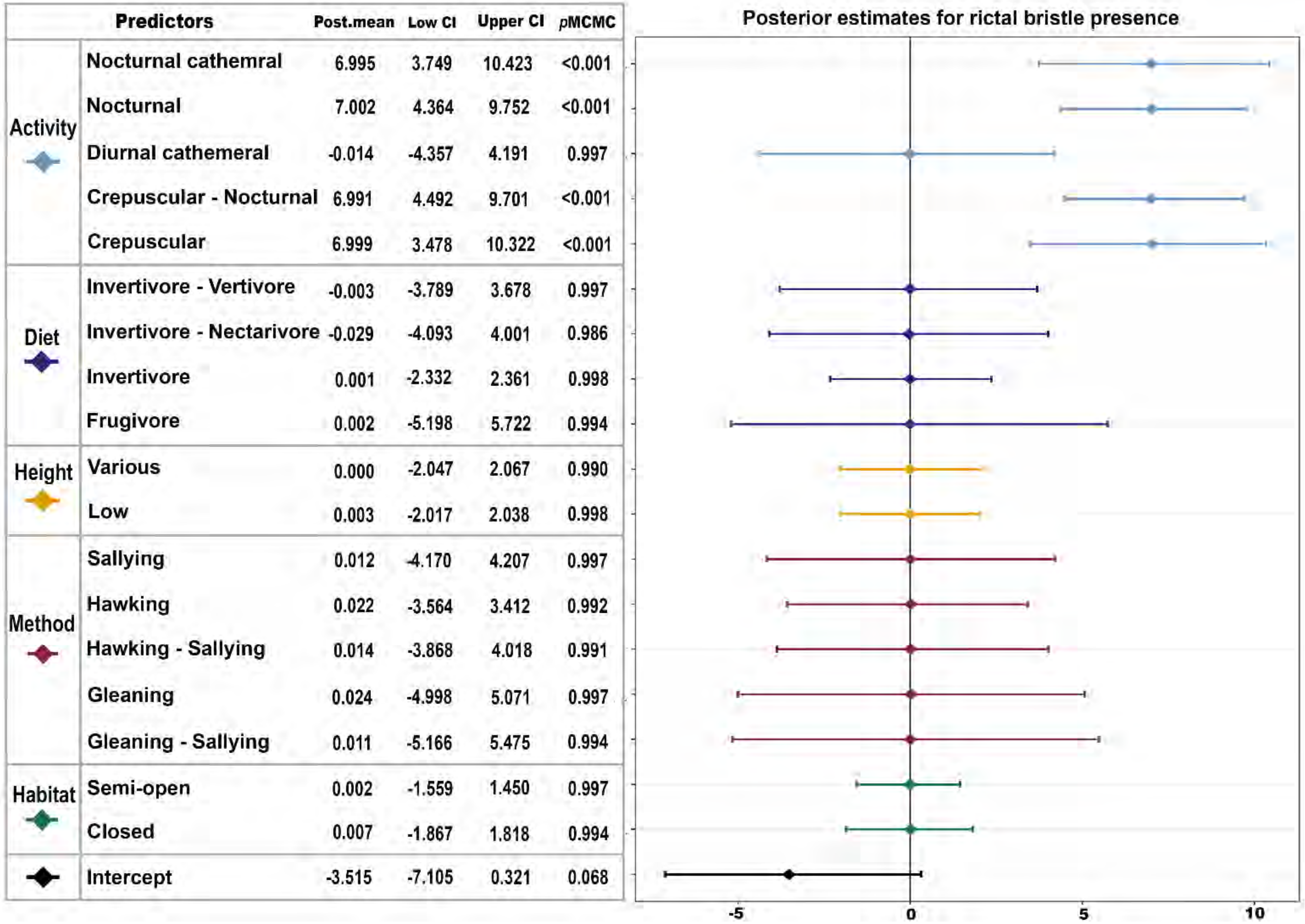


Predictors		Post.mean	Low CI	Upper CI	pMCMC
Shape	Full	-0.019	-0.048	0.011	0.221
	Base	-0.010	-0.034	0.014	0.413
Method	Scanning	0.021	-0.154	0.188	0.807
	Sallying - Plunge diving	-0.034	-0.155	0.087	0.575
	Sallying	0.019	-0.013	0.050	0.239
	Probing	-0.014	-0.059	0.030	0.538
	Lunging	0.017	-0.049	0.085	0.604
	Hawking	0.013	-0.030	0.055	0.552
	Hawking - Sallying	0.035	-0.005	0.073	0.078
	Gleaning - Sallying	0.052	0.021	0.083	0.001
	Gleaning - Hawking	-0.003	-0.022	0.015	0.713
Diet	Vertivore - Invertivore	0.024	-0.076	0.124	0.636
	Omnivore	0.033	-0.044	0.113	0.415
	Invertivore - Vertivore	0.036	-0.045	0.115	0.383
	Invertivore - Nectarivore	0.083	-0.015	0.177	0.094
	Invertivore - Granivore	0.034	-0.046	0.118	0.414
	Invertivore - Frugivore	0.038	-0.038	0.119	0.347
	Invertivore - Aquatic vertivore	0.079	-0.014	0.167	0.090
	Invertivore	0.076	-0.005	0.159	0.070
	Herbivore - Invertivore	0.005	-0.241	0.242	0.962
	Herbivore - Frugivore	-0.035	-0.312	0.250	0.803
	Granivore - Invertivore	0.025	-0.061	0.107	0.559
	Frugivore - Nectarivore	0.033	-0.055	0.121	0.453
	Frugivore - Invertivore	0.014	-0.067	0.090	0.727
	Frugivore - Granivore	0.031	-0.055	0.119	0.489
	Frugivore	0.050	-0.035	0.131	0.245
	Aquatic invertivore-Invertivore	0.026	-0.083	0.134	0.639
Activity	Nocturnal cathemeral	-0.045	-0.135	0.044	0.316
	Nocturnal	-0.003	-0.090	0.081	0.949
	Diurnal cathemeral	-0.006	-0.061	0.045	0.814
	Crepuscular - Nocturnal	-0.009	-0.093	0.076	0.832
	Crepuscular	-0.053	-0.109	0.003	0.063
	Intercept	0.040	-0.123	0.207	0.635

Figure 6-2. **Caterpillar plot representing the posterior mean** (post. mean) **estimates and the 95% credible intervals** (CI) **for each ecological predictor in the normalised rictal bristle length model in all species**. The model intercept is in black, while the activity period is in blue, the diet is in purple, the foraging method is in dark magenta and shape is in grey, of which the control categories were diurnal, frugivore-herbivore, gleaning, and unbranched, respectively.

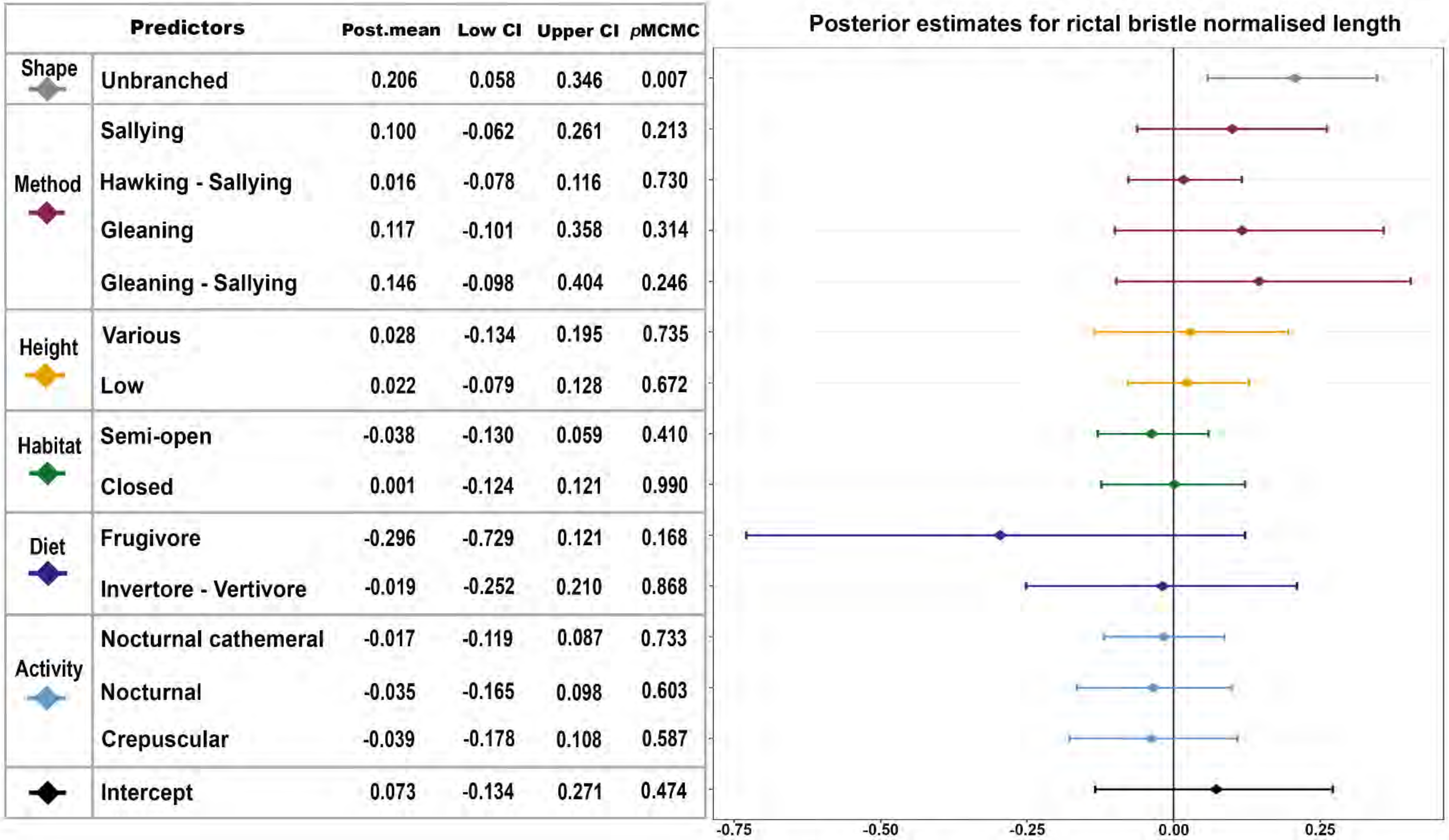
6.3.2 Caprimulgiformae species

Within the Caprimulgiformae, the activity period was the only predictor of the presence of rictal bristles (Figure 6-3). Nocturnal cathemeral (*p*MCMC<0.001), nocturnal (*p*MCMC<0.001), crepuscular-nocturnal (*p*MCMC<0.001), and crepuscular (*p*MCMC<0.001) species were significantly more likely to have rictal bristles present than diurnal species, which all had no rictal bristles (Figure 6-3). Foraging method, foraging height, habitat density and diet were not significantly predicting the presence of rictal bristles (Figure 6-3). None of the ecological traits was significantly predicting the normalised rictal bristle length. However, rictal bristle shape significantly predicted on normalised rictal bristle length, where unbranched rictal bristles (*p*MCMC=0.007) were significantly longer than branched rictal bristles (the morphotype 'branched at the base' were not present within the Caprimulgiformae dataset) (Figure 6-4).



Predictors		Post.mean	Low CI	Upper CI	*p*MCMC
Activity ◆	Nocturnal cathemral	6.995	3.749	10.423	<0.001
	Nocturnal	7.002	4.364	9.752	<0.001
	Diurnal cathemeral	-0.014	-4.357	4.191	0.997
	Crepuscular - Nocturnal	6.991	4.492	9.701	<0.001
	Crepuscular	6.999	3.478	10.322	<0.001
Diet ◆	Invertivore - Vertivore	-0.003	-3.789	3.678	0.997
	Invertivore - Nectarivore	-0.029	-4.093	4.001	0.986
	Invertivore	0.001	-2.332	2.361	0.998
	Frugivore	0.002	-5.198	5.722	0.994
Height ◆	Various	0.000	-2.047	2.067	0.990
	Low	0.003	-2.017	2.038	0.998
Method ◆	Sallying	0.012	-4.170	4.207	0.997
	Hawking	0.022	-3.564	3.412	0.992
	Hawking - Sallying	0.014	-3.868	4.018	0.991
	Gleaning	0.024	-4.998	5.071	0.997
	Gleaning - Sallying	0.011	-5.166	5.475	0.994
Habitat ◆	Semi-open	0.002	-1.559	1.450	0.997
	Closed	0.007	-1.867	1.818	0.994
◆	Intercept	-3.515	-7.105	0.321	0.068

Figure 6-3. Caterpillar plot representing the posterior mean (post. mean) **estimates and the 95% credible intervals** (CI) **for each ecological predictor in the rictal bristle presence model in Caprimulgiformae**. The model intercept is in black, while the habitat density is in green, the foraging method is in dark magenta, the foraging height is in yellow, the diet is in purple and the activity period is in blue, of which the control categories were open, hawking, high, nectarivore-invertivore.

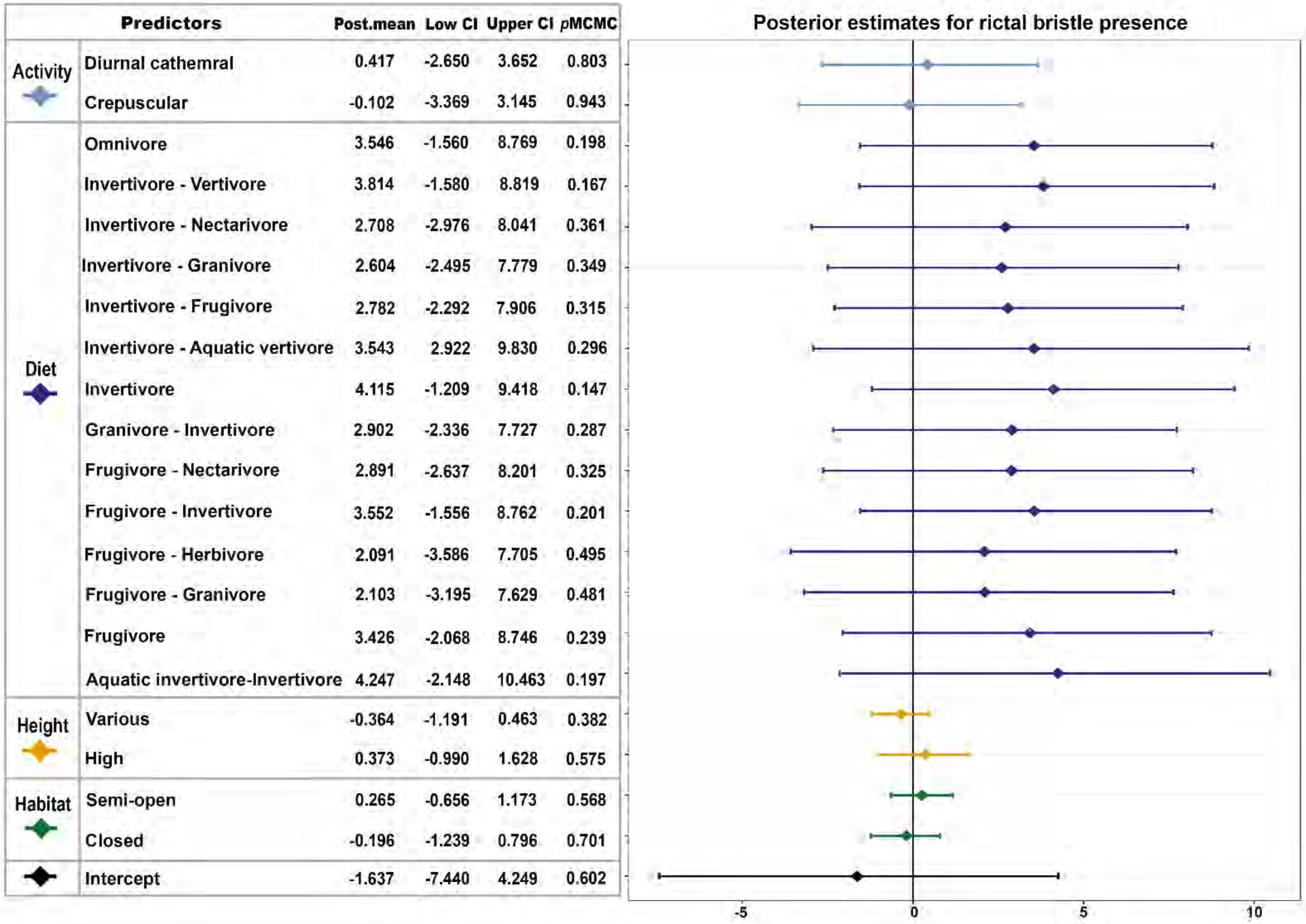


	Predictors	Post.mean	Low CI	Upper CI	*p*MCMC
Shape ◆	Unbranched	0.206	0.058	0.346	0.007
Method ◆	Sallying	0.100	-0.062	0.261	0.213
	Hawking - Sallying	0.016	-0.078	0.116	0.730
	Gleaning	0.117	-0.101	0.358	0.314
	Gleaning - Sallying	0.146	-0.098	0.404	0.246
Height ◆	Various	0.028	-0.134	0.195	0.735
	Low	0.022	-0.079	0.128	0.672
Habitat ◆	Semi-open	-0.038	-0.130	0.059	0.410
	Closed	0.001	-0.124	0.121	0.990
Diet ◆	Frugivore	-0.296	-0.729	0.121	0.168
	Invertore - Vertivore	-0.019	-0.252	0.210	0.868
Activity ◆	Nocturnal cathemeral	-0.017	-0.119	0.087	0.733
	Nocturnal	-0.035	-0.165	0.098	0.603
	Crepuscular	-0.039	-0.178	0.108	0.587
◆	Intercept	0.073	-0.134	0.271	0.474

Figure 6-4. **Caterpillar plot representing the posterior mean** (post. mean) **estimates and the 95% credible intervals** (CI) **for each ecological predictor in the normalised rictal bristle length model in Caprimulgiformae**. The model intercept is in black, while the activity period is in blue, the diet is in purple, the habitat density is in green, the foraging height is in yellow, the foraging method is in dark magenta and shape is in grey, of which the control categories were crepuscular-nocturnal, invertivore, open, high, hawking, and branched, respectively.

6.3.3 Passeriformes species

Within the Passeriformes, none of the ecological traits significantly predicted the presence of the rictal bristles (Figure 6-5). Although foraging method was determined as non-significant fixed effect (6.2.2) for the rictal bristle presence model, foraging method, alongside diet, both significantly predicted normalised rictal bristle length (Figure 6-6). Specifically, rictal bristles were longer for species that glean and sally (*p*MCMC=0.002) compared to those that glean only. Frugivore (*p*MCMC=0.024) and invertivore (*p*MCMC=0.004) species had also longer rictal bristles than frugivore-invertivore (Figure 6-6). Period of activity, foraging height and habitat density did not predict the normalised rictal bristle length (Figure 6-6).



Predictors		Post.mean	Low CI	Upper CI	*p*MCMC
Activity ◆	Diurnal cathemral	0.417	-2.650	3.652	0.803
	Crepuscular	-0.102	-3.369	3.145	0.943
Diet ◆	Omnivore	3.546	-1.560	8.769	0.198
	Invertivore - Vertivore	3.814	-1.580	8.819	0.167
	Invertivore - Nectarivore	2.708	-2.976	8.041	0.361
	Invertivore - Granivore	2.604	-2.495	7.779	0.349
	Invertivore - Frugivore	2.782	-2.292	7.906	0.315
	Invertivore - Aquatic vertivore	3.543	2.922	9.830	0.296
	Invertivore	4.115	-1.209	9.418	0.147
	Granivore - Invertivore	2.902	-2.336	7.727	0.287
	Frugivore - Nectarivore	2.891	-2.637	8.201	0.325
	Frugivore - Invertivore	3.552	-1.556	8.762	0.201
	Frugivore - Herbivore	2.091	-3.586	7.705	0.495
	Frugivore - Granivore	2.103	-3.195	7.629	0.481
	Frugivore	3.426	-2.068	8.746	0.239
	Aquatic invertivore-Invertivore	4.247	-2.148	10.463	0.197
Height ◆	Various	-0.364	-1.191	0.463	0.382
	High	0.373	-0.990	1.628	0.575
Habitat ◆	Semi-open	0.265	-0.656	1.173	0.568
	Closed	-0.196	-1.239	0.796	0.701
◆	Intercept	-1.637	-7.440	4.249	0.602

Figure 6-5. **Caterpillar plot representing the posterior mean** (post. mean) **estimates and the 95% credible intervals** (CI) **for each ecological predictor in the rictal bristle presence model in Passeriformes**. The model intercept is in black, while the habitat density is in green, the foraging height is in yellow, the diet is in purple, and the activity period is in blue, of which the control categories were open, low, granivore, and diurnal, respectively.

	Predictors	Post.mean	Low CI	Upper CI	*p*MCMC
Shape ◆	Unbranched	0.005	-0.031	0.041	0.768
	Branched	0.017	-0.036	0.067	0.533
Method ◆	Sallying	0.013	-0.030	0.055	0.559
	Probing	-0.011	-0.059	0.035	0.648
	Lunging	0.035	-0.103	0.180	0.625
	Hawking	0.041	-0.021	0.101	0.188
	Hawking - Sallying	0.042	-0.006	0.095	0.099
	Gleaning - Sallying	0.059	0.022	0.095	0.002
	Gleaning - Hawking	-0.003	-0.023	0.017	0.743
Height ◆	Low	0.001	-0.018	0.020	0.876
	High	-0.004	-0.033	0.023	0.779
Habitat ◆	Semi-open	0.006	-0.013	0.025	0.569
	Open	-0.001	-0.023	0.023	0.932
Diet ◆	Omnivore	0.021	-0.008	0.049	0.149
	Invertivore - Vertivore	0.025	-0.010	0.059	0.160
	Invertivore - Nectarivore	0.063	-0.002	0.131	0.066
	Invertivore - Granivore	0.017	-0.017	0.055	0.345
	Invertivore - Frugivore	0.019	-0.008	0.046	0.172
	Invertivore-Aquatic vertivore	0.066	0.000	0.135	0.057
	Invertivore	0.066	0.019	0.111	0.004
	Granivore - Invertivore	0.012	-0.033	0.056	0.609
	Frugivore - Nectarivore	0.025	-0.021	0.072	0.301
	Frugivore - Herbivore	-0.010	-0.100	0.086	0.828
	Frugivore - Granivore	0.020	-0.025	0.064	0.378
	Frugivore	0.042	0.006	0.078	0.024
	Aquatic invertivore-Invertivore	0.016	-0.063	0.102	0.699
Activity ◆	Diurnal cathemeral	-0.015	-0.087	0.056	0.687
	Crepuscular	-0.010	-0.142	0.004	0.059
◆	Intercept	0.092	-0.030	0.217	0.150

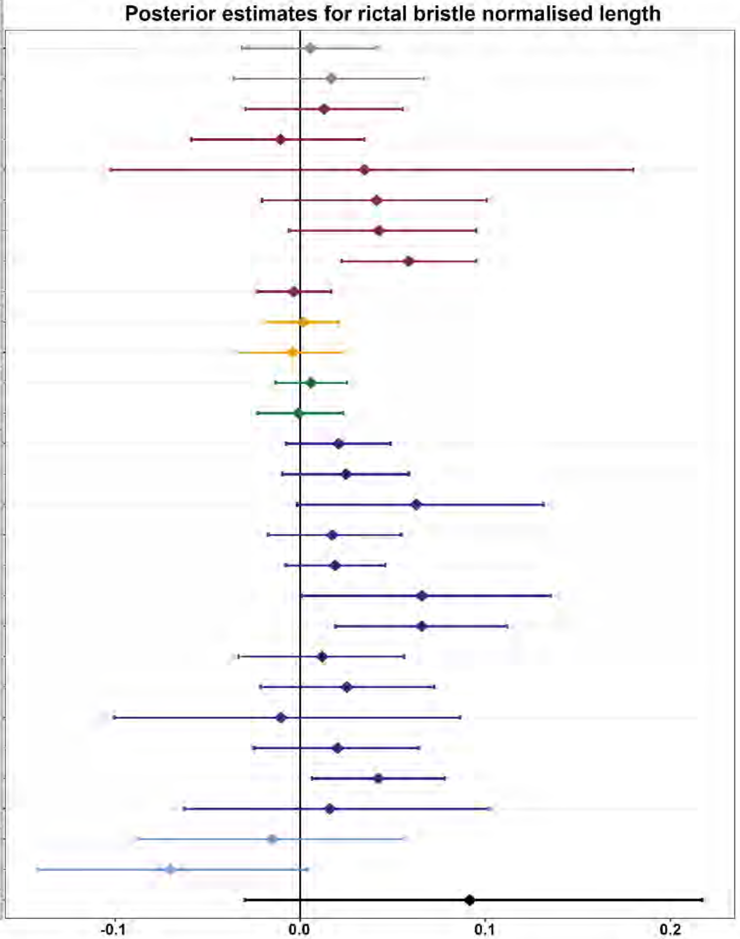


Figure 6-6. Caterpillar plot representing the posterior mean (post. mean) **estimates and the 95% credible intervals** (CI) **for each ecological predictor in the normalised rictal bristle length model in Passeriformes**. The model intercept is in black, while the activity period is in blue, the diet is in purple, the habitat density is in green, the foraging height is in yellow, the foraging method is in dark magenta and shape is in grey, of which the control categories were diurnal, frugivore-invertivore, semi-open, various, gleaning, and unbranched, respectively.

6.4 Discussion

Our results suggest that some species-specific ecological traits are associated with rictal bristle presence and length, independent of phylogenetic relatedness. Specifically, nocturnality and certain foraging methods (sallying-plunge diving, scanning, and gleaning-sallying) are both contributing factors to having long, present rictal bristles. Diet may also be associated with normalised rictal bristle length, whereas habitat density and foraging height were not associated with rictal bristle presence and normalised length in any of the models explored here.

Nocturnality

For both the full dataset and for Caprimulgiformae only, species associated with the nocturnality spectrum (including nocturnal-cathemeral to obligate nocturnal) were more likely to possess rictal bristles than diurnal species. For example, within the Caprimulgiformae, diurnal Apodiformes (Hemiprocnidae, Apodidae and Trochilidae) did not possess rictal bristles, while the nocturnal-cathemeral to obligate nocturnal strisoreans (Caprimulgidae, Podargidae, Aegothelidae, Steatornithidae, and Nyctibiidae) possessed rictal bristles, that were also relatively long (Chapters 3 and 6). Apodiformes evolved from Caprimulgiformes, reverting to diurnality (Hackett *et al.*, 2008); therefore, the loss of rictal bristles could have coincided with the shift from the crepuscular/nocturnal life-history trait to the diurnal life-history. This would also lend support to the suggestion that rictal bristles may play a tactile role in nocturnal species (Cunningham *et al.*, 2011) since more tactile bristles (indicated by more mechanoreceptors around the bristle follicle) are prevalent in nocturnal species such as kiwis, morepork, hihi, oilbird, and frogmouths (Cunningham *et al.*, 2011; Delaunay *et al.*, 2020; Chapter 3). The shift in the environment of Apodidae (i.e. shifting from a nocturnal environment to diurnal one) and the disappearance of the rictal bristles suggests that rictal bristles may carry a cost, and be genotype-specific and environment-dependent (Murren et al., 2015); evidencing that rictal bristles are phenotypic rather than plastic. If so, this could mean that rictal bristles are less beneficial in a diurnal environment. However, to understand the cost of the trait, genotypes need to be considered (DeWitt et al., 1998).

However, rictal bristles were also present in species from each category of the activity period spectrum, including diurnal species. Unlike the Caprimulgiformae, the Passeriformes order is predominantly composed of diurnal species, although a few species are diurnal-cathemeral and crepuscular (Billerman *et al.*, 2020). Passeriformes species from each of these activity period categories did possess rictal bristles, which could explain why activity period was not associated with rictal bristle presence in the Passeriformes. Nevertheless, ~95% of all Passeriformes species were not recorded here. Sampling these absent species could lead to finding more diurnal cathemeral and crepuscular species having rictal bristles, and so an association might emerge then. Furthermore, the Passeriformes order is widely recognised in terms of its species richness and diversity of morphological characteristics present amongst species; therefore, normalised rictal bristle length (if not present) is likely to be influenced by other ecological factors, such as species-specific foraging methods (e.g. glean and sally) and diet (e.g. frugivore, invertivore).

Foraging methods

Foraging methods were also associated with rictal bristles. Specifically, in the full dataset, sallying-plunge diving and scanning species were more likely to have rictal bristles present than dabbling species. Foraging method is a complex trait, which involves the search, assessment, pursuit, and capture of a prey item (Kramer, 2001). Therefore, identifying which aspect of a species foraging method serves as a significant predictor for rictal bristles remains challenging and merits further investigation, especially by widening the number of species sampled.

i. Sallying- plunge-diving method

The species that foraged by both sallying and plunge-diving in this study are all species of the Alcedinidae family (kingfishers), and all but one (*Ceyx rufidorsa*) had rictal bristles. Species that only plunge dive to feed (e.g. Alcedinidae) overcome drag and buoyancy when transitioning from air to water by diving from height (Crandell *et al.*, 2019). This method evolved in aerial foragers to facilitate entry into the water, and subsequently driving morphological adaptation towards the improvement of dive efficiency and damage avoidance upon entry into the water (Crandell *et al.*, 2019). Therefore, bristle absence in plunge divers is likely due to potential damage sustained on entry.

The Alcedinidae family is predominantly composed of terrestrial and aquatic species (Crandell et al., 2019; Billerman et al., 2020) and comprise of i) small terrestrial kingfishers that feed mainly on insects, ii) larger terrestrial kingfishers that feed mainly on small vertebrates (lizards, mammals and birds), and iii) aquatic kingfishers that feed primarily on fish (Billerman *et al.*, 2020). Some terrestrial kingfishers forage by sallying and plunge-diving, others only sally on terrestrial substrates, and the aquatic kingfisher species plunge dive (Crandell et al., 2019; Billerman et al., 2020). According to two studies (Sibley and Ahlquist, 1990; Moyle et al., 2007), the subfamily Alcedininae (river kingfishers) is likely to be the basal branch in the kingfishers' phylogeny. Within this subfamily, three species were measured in this study – all three without rictal bristles. Of these three species, one species is known only to sally (*Corythornis madagascariensis*), one forages by plunge-diving (*Corythornis cristatus*), while the remaining species sallies and plunge dives for prey (*Ceyx erithaca*). Moreover, the Cerylinae (water kingfishers) subfamily, of which the species measured in this study solely plunge dive to feed (*Ceryle rudis, Chloroceryle aenea,* and *Megaceryle torquata*) did not have rictal bristles, whereas six of the eight species measured belonging to the last Alcedinidae subfamily, the Daceloninae (forest kingfishers), all of which sally or sally and plunge dive, did have rictal bristles present. This could suggest that during their evolution, rictal bristles disappeared in plunge diving only species, in favour of hydrodynamic modifications, and subsequently reappeared within the Alcedinidae, as possibly being beneficial to sallying, and sallying-plunge diving species. Although, the loss of rictal bristles in the Alcedinidae has previously been documented in Chapter 6, rictal bristle presence was conserved from the ancestral character state in the most recent common ancestor of the Alcedinidae family to the Daceloninae. More species of the basal branch Alcedininae should be measured to verify the evolution of the rictal bristle presence within the Alcedinidae, especially if the ancestral character state in the most recent common ancestor of the Alcedinidae is bristle presence.

ii. Scanning method

The other foraging method associated with rictal bristle presence was scanning, which is performed by some Accipitridae (hawks and eagles) and Strigiformes (owls). Strigiformes are close relatives of the Caprimulgiformes and more distant from the Accipitridae (Sibley and Ahlquist, 1990; Galeotti and Rubolini, 2004). Owls do share several morphological and ecological traits with hawks and eagles due to their predatory behaviour (Galeotti and Rubolini, 2004) and rictal bristles may represent one of these shared morphological traits since all Strigidae and Tytonidae species and half of the Accipitridae species measured had rictal bristles. Tytonidae and Strigidae forage in extremely low light conditions (nocturnal spectrum from nocturnal cathemeral to obligate nocturnal) (Billerman *et al.*, 2020), while Accipitridae include mainly diurnal species, although there are some diurnal cathemeral (e.g. *Elanus axillaris*), crepuscular (e.g. *Aviceda madagascariensis*) and nocturnal species (e.g. *Elanus scriptus*). Küster (1905) found mechanoreceptors in the surroundings of rictal bristle follicles in some owl species (*Bubo bubo, Asio flammeus, Athene noctua, Strix aluco*), and I found this to also be the case in *Tyto alba* (Chapter 4), indicating that they are likely to be vibrotactile sensors. Therefore, in low-light condition, rictal bristles could play a role in orienting the beak, so they might be able to accurately discriminate the prey caught in their talons. Küster (1905) even suggested that rictal bristles may be able to sense sound waves (Lederer, 1972), if so, rictal bristles could play a role in environmental scanning for sounds of prey but further research is needed.

Scanning typically involves head movements to visually scan for prey items (O'Rourke *et al.*, 2010). Bristles might be able to detect movements of the head, and use this to either guide the rotations or provide information about proprioception. In Chapter 3 of this study, I suggested that rictal bristles of diurnal species might have a reduced or absent tactile function, although stretch receptors in the skin still might detect movement of the bristles, even if mechanoreceptors are absent. Therefore, the rictal bristles may play a role in head orientation during scanning, even in diurnal species, or those without bristle follicle mechanoreceptors.

iii. Gleaning-sallying method

Species that forage by gleaning and sallying in the full dataset, and just the Passeriformes, had longer bristles than species that foraged only by gleaning. Species that hunt prey by sallying tend to ”sit and wait”, therefore catching their prey with a short attack, without gliding or hovering (Remsen and Robinson, 1990). Therefore, this short attack might increase collision chances between flying foreign items, such as food times and vegetation. These species (e.g. species of the Tyrannidae family), often additionally glean (i.e. foraging without being in flight) on prey items perched on those same branches and leaves (Verbeek, 2015). However, species that forage by only gleaning tend to move around on foot (Forstmeier and Keßler, 2001) and rictal bristles are shorter in nocturnal cathemeral species that aerially forage in open habitats (Chapter 3).

Lederer (1972) demonstrated that rictal bristle did not play a tactile role in Tyrant flycatchers and, rather, protected the eye. Longer rictal bristles are, therefore, more likely to protect the eyes from dislodged items and foliage during foraging. In contrast, species that hawk tend to forage in more open habitats (Verbeek, 2015), and are less likely to collide with foreign items and so selection for bristles is weak. Sallying species are more likely to collide with foreign items and might have less time to manoeuvre to avoid collisions, therefore retaining functional bristles for protection of the eye. Species that both sally and glean are likely to encounter collisions between their eyes and food items (e.g. legs, wings), and vegetation, which could explain why these species had longer rictal bristles protecting their eyes compared to only gleaning species. Rictal bristles may be able to detect prey coming into the mouth during sallying, and allow the bird to make adjustments in the orientation of its gape. Alternatively, rictal bristles may be able to detect upcoming collisions with vegetation and help to guide avoidance behaviours and eye closing. If this were the case, then the detection and responses to rictal bristle touch needs to be rapid.

Herbst corpuscles vary in size and layering amongst species, which affects their optimal response frequency; however, overall, they have very similar sensitivity ranges, i.e. 40-50 Hz (except in goose in which they are tuned to higher frequencies, 130-170 Hz) (Quindlen-Hotek et al., 2020). However, visual flicker fusion frequency in diurnal species, such as pied flycatcher (*Ficedula hypoleuca*), collared flycatcher (*Ficedula albicollis*), and blue tit (*Cyanistes caeruleus*), average up to 145Hz (Boström et al., 2016). Therefore, visual responses are probably much faster than tactile responses. Faster responses to potential collisions and accurate orientation of the beak is, therefore, more likely to come from visual information. However, flicker fusion frequency is lower in nocturnal species than diurnal species, e.g. optimal frequency recorded were 50Hz in the little owl (*Athene noctua*), 67.5 in short-eared owl (*Asio flammeus*) (Bornschein and Tansley, 1961; Porciatti et al., 1989). Therefore, longer rictal bristles may still be beneficial and supplement for collision detection responses in these nocturnal species.

Diet

Diet was also associated with the normalised rictal bristle length, but only in the Passeriformes. Specifically, rictal bristles of Passeriformes were longer in frugivorous and insectivorous species, compared to frugivore-invertivores species. Within the Passeriformes, all the invertivores measured foraged by aerial feeding (hawking and sallying in the air) and terrestrial feeding (gleaning on the ground, on branches and trunks), while gleaning was the only feeding behaviour reported for the frugivores measured. All frugivore-invertivore species measured were Corvidae species. Frugivore species feed on all types of fruits, from soft to hard, and pluck fruits attached to the plant or fallen on the ground (Rico-Guevara *et al.*, 2019). Rictal bristles might be able to discriminate fruits from other plant structures, as well as detecting ripe textured fruit. The bristles may also guide the orientating of the head towards the target fruit. The rictal bristles could protect their eyes from foliage, especially if foraging in low-light condition such as in the oilbird (Billerman *et al.*, 2020). Invertivore species also feed on a variety of different invertebrates, which are morphologically and behaviourally diverse (Rico-Guevara *et al.*, 2019), hence these species exhibit different feeding methods, including hawking, sallying, gleaning and probing. Avian diets often vary seasonally and some species exhibit significant variation in their diet (Rico-Guevara *et al.*, 2019), hence the mixed frugivore-invertivore category used in this study. Two distinct feeding modes might be functionally and morphologically challenging, as trade-offs are commonly found between traits, i.e. enhancing one feeding mode over another (Rico-Guevara *et al.*, 2019). Dietary flexibility might, therefore, influence morphological and functional constraints and could also be responsible for the observed rictal bristle length difference between frugivore and invertivore specialists and more generalists (frugivore-invertivore) species - with longer rictal bristles in specialists and shorter ones in generalists.

Some authors argue that diet and foraging method are not independent, and the way the food is obtained might be more relevant to feeding behaviour than diet (Rico-Guevara *et al.*, 2019), and so could be more relevant to rictal bristle presence and morphology. This would concur with the findings of this present study, as foraging methods have a significant association with bristle presence in all species, whereas diet only affects normalised rictal bristle length in the Passeriformes.

Conclusions

Rictal bristle presence and length are associated with nocturnality, as well as some aspects of foraging, such as foraging method and diet. This study supports findings from Chapter 3 and demonstrates that species foraging in low light conditions might have tactile rictal bristles, while species foraging in lighter conditions and during daylight might have a reduced tactile function. Additionally, rictal bristles might have been lost and reduced in length in bird species that dive, hawk and glean, as well as those species that have a flexible diet. Therefore, I suggest that birds foraging in in the dark are most likely to have long rictal bristles that may play a role in eye protection - either physically or by allowing avoidance responses, such as eye closing. This is supported by our evidence that rictal bristles may be absent in species that forage at high-speeds - in open air or water - which might be to prevent damage to the bristles, reduce drag and because selection for their retention is weak. However, more research is needed to confirm these suggestions.

Chapter 7

Describing candidate somatosensory brain areas in *Caprimulgus pectoralis* and *Caprimulgus vexillarius*

Chapter summary:

Nocturnal species have specialised senses for guiding foraging and navigation in the dark, this includes stereoscopic visual depth perception, as well as improved hearing and touch. Brain size and shape differ within species, and these differences are often associated with sensory specialisations. Caprimulgiform species are mostly nocturnal and possess prominent rictal bristles on their upper beak. It is not yet clear how sensory signals from the rictal bristles are presented in the brain, although the trigeminal system, cerebellum, optic tectum and the Wulst are all likely candidate structures to be involved with somatosensory processing. Within this chapter, I will describe associated mechanosensory brain areas in two afro-tropical nightjar species *C. pectoralis* and *C. vexillarius*, which forage nocturnally and diurnally, respectively. Brain and Wulst size were larger in *C. vexillarius* than *C. pectoralis*. *C. pectoralis* had a larger optic tectum, especially in layer 13, which is associated with tactile and visual processing. Comparing neuroanatomy with other species did not show clear associations with sensing or foraging, so these relationships are likely to be complex. Brain anatomy varies considerably between species, and it is important to understand phylogenetic differences as well as functional adaptations. Future work should compare many species and conduct quantitative phylogenetic comparisons.

7.1 Introduction

Nocturnal species have specialised senses for guiding foraging and orientation in the dark; this includes enhanced visual sensitivity, such as stereopsis within their binocular field (depth perception) (Iwaniuk and Wylie, 2006), as well as improved hearing and touch (Le Duc and Schöneberg, 2016). In birds, brain size and shape vary between species (Walsh and Milner, 2011), and are closely associated with visual, auditory and somatosensory systems (Nomura and Izawa, 2017). Visual and touch processing are likely to be carried out through the spinothalamic tract to the telencephalon - passing through tectofugal pathways in the midbrain (e.g. layer 13, or stratum griseum central in the optic tectum) to nuclei of the thalamus (nucleus rotundus (Rt) and the principal sensory trigeminal nucleus (PrV)), and on to the entopallium, the nucleus basorostralis (Bas) or the Wulst in the telencephalon (see Chapter 2 for more specific details). Indeed, optic lobes, such as the optic tectum in the midbrain, are primarily associated with visual information, which is projected from layer 13 to Rt in the thalamus, which in turn projects into the entopallium of the telencephalon (Reiner and Karten, 1982; Wylie et al., 2009; Fernández et al., 2020).

Bas and PrV, however, receive tactile information from the beak, and are particularly enlarged in tactile foragers such as waterfowl, kiwis and probing shorebirds (Gutiérrez-Ibáñez et al., 2009; Cunningham et al., 2013; Iwaniuk and Wylie, 2020). Integrating between multiple senses (i.e. vision and touch) is thought to be carried out in the Wulst in birds, with principal areas receiving visual or tactile signals (Delius and Bennetto, 1972; Karten et al., 1973; Pettigrew, 1979; Funke, 1989; Wild and Williams, 2000). The Wulst size, shape, position and organisation (i.e. degree of lamination of the lamina formed by sheets of bimodal neurons) varies between species. As yet, its enlargement is likely to be associated with an enhanced visual system, such as stereopsis, and is especially enlarged in owls (Medina and Abellán, 2009; Walsh and Milner, 2011). However, as a higher degree of lamination in the brain is associated with more topographical maps, differences in the Wulst could also reflect functional differences (Iwaniuk and Wylie, 2006). Therefore, a larger Wulst could be expected in species that rely on both tactile and visual cues rather than visual cues only. Furthermore, many mammalian tactile specialists have a topographic whisker map within the primary somatosensory cortex (Woolsey *et al.*, 1975). As the Wulst is thought to be functionally similar to the mammalian cortex (Pettigrew, 1979), a topographic mechanosensory specialisation in their avian telencephalon, specifically in the Wulst, might be expected in tactile species.

This study focuses on two nightjar species belonging to the Caprimulgiformes order, *Caprimulgus pectoralis* and *Caprimulgus vexillarius,* of which the brain morphology and anatomy has not yet been described. These two species differ in ecology, notably in the time of activity - with *C. vexillarius* being nocturnal cathemeral (i.e. starts foraging from late afternoon into the night) and *C. pectoralis* being an obligate nocturnal. They also differ in the anatomy of their rictal region, which has previously been described in Chapter 3. Indeed, although both species have prominent rictal bristles, their follicle anatomy is different. *C. vexillarius* did not have any mechanoreceptors around the rictal bristle follicles (mechanoreceptor number = 0), while many mechanoreceptors were found around the bristle follicles of *C. pectoralis* (mechanoreceptor number = 10) (Table 3-4, Chapter 3). Therefore, I predict that *C. pectoralis* would have more mechanosensory adaptations in the brain, whereas *C. vexillarius* would have more visual adaptations.

This study will address the following hypothesis outlined in Chapter 2:

- **Rictal bristle presence and morphology will be associated with mechanosensory associated brain areas**

Species having mechanoreceptors in the surroundings of their rictal bristle follicles might have a larger rostral fraction of the Wulst as well as other somatosensory areas, such as the thalamic nuclei (i.e. Bas and PrV).

Specifically, this study will determine if the presence and morphology of rictal bristles can be positively associated with the Wulst volume (i.e. indicating a potential reliance on mechanosensory cues) and the relative volume of the whole brain (indicating potential association of more than the Wulst structures, e.g. cerebrotypes), and negatively correlated with the volume of the optic tectum (potentially relying less reliance on vision). In order to do that, the brain morphology, composition and Wulst relative volume of two nightjar species, *C. pectoralis* and *C. vexillarius*, will be qualitatively described and compared to other nocturnal species presented in Iwaniuk and Wylie (2006), including *Eurostopodus argus, Podargus strigoides, Tyto alba, Ninox boobook, Steatornis caripensis and Aegotheles insignis*. The associations of sensory areas (somatosensory and visual) with foraging traits, rictal bristle length and mechanoreceptor number (described in Chapters 3 and 4) in the aforementioned species will be discussed. The gross neuroanatomy of *C.*

pectoralis and *C. vexillarius* will be compared to *E.* argus from Iwaniuk and Wylie (2006) and to the optic lobe anatomy of the pigeon (*Columbia livia*) from Wylie *et al.* (2009). Finally, the association between the bristle presence (data from Chapters 5 and 6) and the relative volume of the whole brain volume, the optic tectum and the Wulst (neuroanatomy data supplied by Iwaniuk *et al.* (2008)) will be considered in 45 species (include 33 species without rictal bristles and 12 species with rictal bristles) from different orders to account for phylogenetic relatedness.

7.2 Material and methods

7.2.1 Samples

Only two species of African nightjars, the fiery-necked nightjar (*Caprimulgus pectoralis*) and the pennant-winged nightjar (*Caprimulgus vexillarius*) were used in this study, since this material is rare and not usually conserved, and these were the only two we could have access to. The two deceased specimens were found by the side of a road in Zambia (Africa). Following their discovery, they were decapitated, preserved in formalin and stored at the University of Sheffield for a number of years before being brought to Manchester Metropolitan University as part of this study. Due to being stored in formalin for a long time, there was some shrinkage of the brain tissue in both specimens.

7.2.2 Brain shape descriptions

C. pectoralis and *C. vexillarius* formalin-fixed heads were dissected by cutting and pulling away the skin to reveal the skull. Photos of the brain orientation within the braincase were taken with a phone camera. The skulls were then cut open with a scalpel to extract the brain. The brain was extracted and cleaned, keeping the majority of the optic nerve and spinal chorda intact. Once removed, both brains were photographed using a digital camera, and brain shape and orientation were qualitatively described. Qualitative comparisons were made between visible regions in the two brains, including the cerebellar foliums, the mesencephalon (optic tectum, midbrain and thalamus), the medulla oblongata, the optic chiasma, the olfactory lobes and the telencephalon of which the anterior part, the Wulst, was noticeable (Figure 7-1). The parts of the mesencephalon and the telencephalon were of particular interest as they are thought to receive somatosensory information.

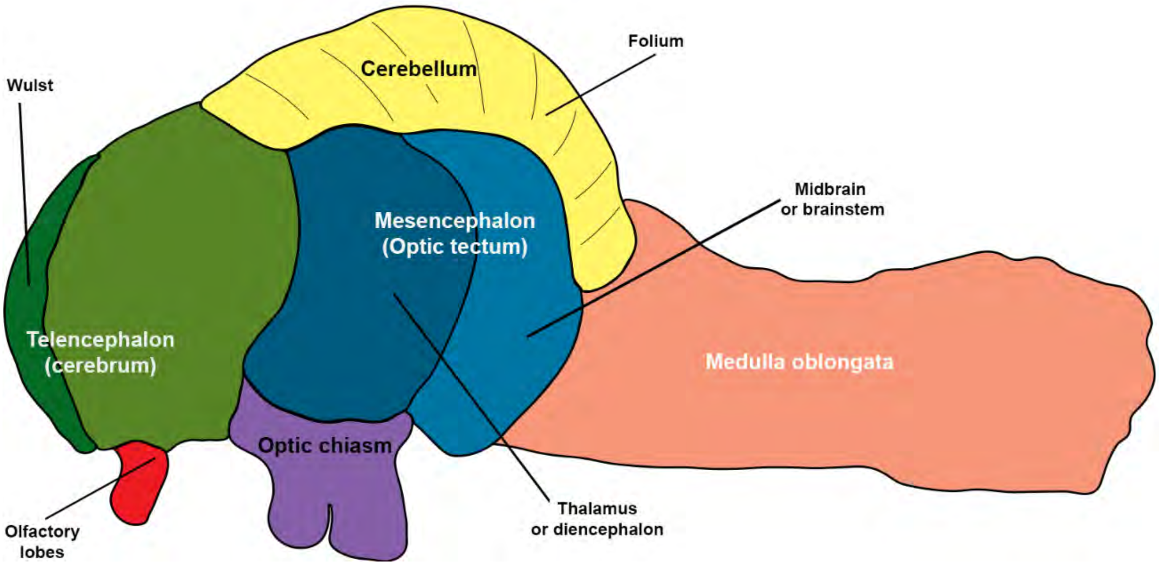


Figure 7-1. Diagram of a nightjar brain (based on *Caprimulgus pectoralis'* brain), illustrating the different brain regions that can be observed after dissection.

Brain length and width were measured for both brains (as indicated in Figure 7-2) from photographs in the longitudinal superior orientation using ImageJ software. Once the brains were dissected and photographed, they were left overnight in 4% PFA (Paraformaldehyde) and then cryoprotected with a solution of 30% sucrose in 0.1M PBS (Phosphate Buffered Saline) until they sunk.

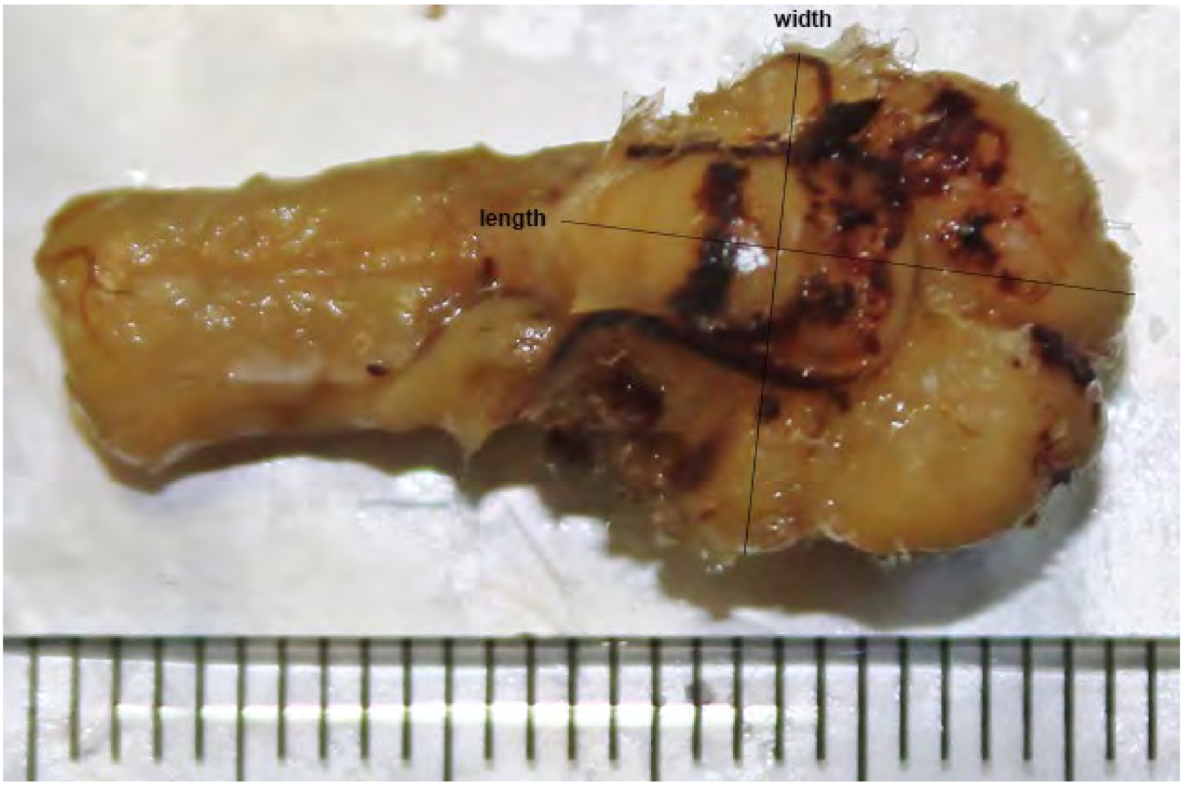


Figure 7-2. **Longitudinal superior photo of *C. pectoralis'* brain** illustrating the width and length measurements were taken (lines). The ruler was used as the scale to calibrate the measurements.

7.2.3 Brain anatomy

Both brains were embedded in Neg 50TM frozen section medium (Richard Allan scientificTM) in a peel-a-way® embedding mould for coronal plane sectioning. Brains were put in the freezer at -80°C for at least an hour to obtain frozen blocks, which were sliced at 50µm with a frozen cryostat microtome (Leica CM 3050) at -19°C. The sections were placed in well-plates with phosphate buffer solution (10% PBS) before being mounted on subbed slides and stained. The sections were allocated to three different staining protocols, including Nissl (to view cell size and number), and staining for myelin (to view nerve cells) and perfused vasculature (to view blood vessels).

Sections were prepared for Nissl staining with a ddH_2O bath, passed through graded baths of ethanol (70, 90, 100 %; 1 min each) and two xylene baths (5min each) and then reversed to ddH_20 bath. Sections were afterwards moved to a Cresyl violet bath for 5 minutes, 70% and 90% for 1 minute, 2x100% ethanol (2min each) and finally two baths of xylene (5 min each) before being coverslipped with DPX mountant for microscopy. For the myelin staining, the slides were submerged in a 100ml ddH_2O and 2ml of osmium bath, until a visible contrast of brown shades were observed in the tissue (after about 2 hours). Three washes of ddH_2O were applied, followed by the mounting and coverslipping process. The perfused vasculature staining process was conducted in the well plates. PBS solution was removed and replaced by a solution of 99ml PBS, 100µl of 10% hydrogen peroxide and 40mg DAB (3,3'-diaminobenzidine). 500µl of the aforementioned solution was pipetted into each well. The sections were over-exposed to the stain by being kept in the solution for 30min. Finally, the sections were rinsed, mounted and coverslipped on subbed slides.

Images of the stained sections were taken on a Zeiss AxioImager M1 Brightfield microscope and AxioCam HMRc using Zeiss Zen Pro imaging software. The myelin and perfused vasculature staining did not show more structure and components details than the Nissl staining, therefore they were not included in the remainder of the study. Nissl staining revealed sections with a good contrast to identify distinguishable brain areas; therefore, only Nissl staining will be reported in this study. However, the Nissl staining also revealed that the two specimens had different quality brain tissue; *C. pectoralis* had a relatively dense brain tissue while *C. vexillarius* had a relatively porous, deteriorated tissue (Figure 7-7). Therefore, it was not possible to conduct quantitative analyses, such as cell counts and sizes, to compare the two species. Since the brains of these two species have never been previously described, this chapter will provide a qualitative overview of the brain anatomy of the two species; focussing on the better-quality *C. pectoralis* specimen. It will also compare gross neuroanatomy to the spotted nightjar (*Eurostopodus argus*) from Iwaniuk and Wylie (2006) and to the optic lobe anatomy of the pigeon (*Columbia livia*) from Wylie *et al.* (2009).

7.2.4 Wulst morphology

Wulst length, width, planar area and shape was extracted for each species. The Wulst was identified from three photographs from an anterior, a lateral right and a lateral left view of the brain of each species (Figure 7-8). The outline of the Wulst areas was manually traced in each image by following the vallecula on the lateral side of the prominence using Zeiss Zen lite software. The photos were calibrated individually using a ruler, and normalised using the brain length and width measurements (Normalisation factor= $\sqrt{\text{Length x Width}}$). Wulst area was calculated in Zen light software. For comparison, the same process was also repeated on lateral photos of the brain of six other avian species, taken from Figure 2 in Iwaniuk and Wylie, 2006, including the spotted nightjar (*Eurostopodus argus*), the tawny frogmouth (*Podargus strigoides*), the barn owl (*Tyto alba*), the Australian boobook (*Ninox boobook*), the Oilbird (*Steatornis caripensis*) and the feline owlet-nightjar (*Aegotheles insignis*). Pairwise Spearman's Rank correlations were conducted between measurements of the brain and Wulst volume (taken from Iwaniuk and Wylie, 2006) and rictal bristle related data (bristle length, bristle number and number of mechanoreceptors; chapter 3) were conducted in R Studio software (R Core team, 2018). Ecological traits, such as habitat type, foraging method, period of activity, were also collected from the Birds of the World website (Billerman *et al.*, 2020) in order to qualitatively discuss their associations with neuroanatomy, since foraging behaviour is the most likely set of traits to be associated with a sensory function (e.g. Wallman and Pettigrew, 1985; Moroney and Pettigrew, 1987; Iwaniuk and Hurd, 2005, and confirmed in Chapter 6).

7.2.5 Association of rictal bristle presence and brain volumes

To better examine the association of rictal bristles and brain volumes over a wider range of species, this study was extended. Thirty-three more species were included to investigate. These thirty-three species had rictal bristle presence data associated with them (recorded in Chapter 5 and 6, see Method section 5.2.1). Brain volume data was taken from Iwaniuk *et al.* (2008), who took digital photographs throughout the brain for every second section, and measured the brain volumes with the public domain NIH Image program (http://rsb.info.nih. gov/nih-image/) , and included volumes of the relative whole brain, the optic tectum (TeO) and the Wulst volume (Table 7-1). Another thirteen species were also included (to give a total of 45 species). These also had brain volume data from Iwaniuk *et al.* (2008), these exact species were absent from my dataset in Chapter 6 dataset, however, I did have data from species of the same genus or family and often bristle absence was obvious (such as from aquatic foragers, such as Spheniscidae or Ardeidae) or presence was clearly visible on BirdsOfTheWorld.org (Billerman et al., 2020) clear (i.e. Strigidae owls). The subsequent dataset, therefore, included 45 species constituting 22 different families across 10 orders, of which 12 species had rictal bristles and 33 had not.

To obtain the normalised relative volume of the whole brain, the Wulst and the TeO, each volume was divided by the species body mass provided by Eltontraits 1.0 dataset (Wilman *et al.*, 2014) (Table 7-1). To account for the phylogenetic relatedness in the analysis, a simulation-based phylogenetic ANOVA (nsim= 1000) with post-hoc comparisons of means among groups (*phylANOVA* (Garland et al., 1993) of the phytools package (Revell, 2012)) was conducted with bristle presence data (presence/absence as binary data). The consensus phylogenetic tree generated for Chapter 5 (see 5.2.2) was be pruned to correspond to the 45 species of the dataset and used here for reducing phylogenetic uncertainty.

Table 7-1. Species brain, Wulst (W) **and optic tectum** (TeO) **volume** (mm^3), **body mass and rictal bristle presence**. Red binary data in bristle presence correspond to the data that has been extrapolated from Chapter 6 and BirdsOfTheWorld.org (Billerman et al., 2020).

Order	Family	Species	Brain	W	TeO	Body Mass	Bristle
Anseriformes	Anatidae	Anas platyrhynchos	5738	572.23	251.48	843.42	0
		Dendrocygna eytoni	4850	499.07	163.65	789.99	0
Caprimulgiformes	Apodidae	Chaetura pelagica	343	16.61	30.47	23.6	0
	Aegothelidae	Aegotheles insignis	1540	363.63	73.64	78.5	1
	Caprimulgidae	Nyctidromus albicollis	910	66.03	36.95	57.84	1
	Nyctibiidae	Nyctibius griseus	1980	176.67	125.58	172.04	1
	Podargidae	Podargus strigoides	5311	1226.89	290.88	308.03	1
	Steatornithidae	Steatornis caripensis	3900	749.53	104.7	408	1
Charadriiformes	Scolopacidae	Calidris minutilla	472	12.66	43.34	22.88	0
		Charadrius vociferus	1073	19.74	130.65	96.44	0
		Limnodromus griseus	1124	33.92	51.12	110.53	0
	Laridae	Sterna hirundo	1593	57.09	121.49	129.15	0
	Charadriidae	Vanellus miles	2686	127.85	205.47	387	0
Columbiformes	Columbidae	Columba livia	2093	187.43	198.29	354.2	0
Galliformes	Ardeidae	Ardea cinerea	8446	520.41	697.78	1443	0
		Bubulcus ibis	4025	220.7	211.02	365.95	0
		Egretta thula	3740	196.41	443.74	371	0
		Nycticorax caledonicus	3360	224.17	268.95	856	0
	Numididae	Numida meleagris	3951	228.52	328.46	1299	0
	Cracidae	Ortalis canicollis	3374	203.83	271.27	539	0
Passeriformes	Corvidae	Garrulus glandarius	3943	448.99	248.9	159.46	1
Psittaciformes	Cacatuidae	Cacatua roseicapilla	6653	676.33	203.44	325.33	0
		Calyptorhynchus funereus	16078	2036.7	307.5	674.72	0
		Nymphicus hollandicus	2339	247.88	81.29	94.61	1
	Psittacidae	Myiopsitta monachus	3697	253.61	156.38	120	0
		Pionus menstruus	5473	408.63	257.95	251	0
		Pyrrhura molinae	4656	497.19	232.93	77.09	0
	Psittaculidae	Agapornis roseicollis	2008	194.32	79.74	53.83	0
		Agapornis personata	2786	204.54	82.57	52.38	0
		Alisterus scapularis	4779	506.55	201.21	232.26	0
		Amazona aestiva	7903	759.2	272.5	451	0
		Aratinga acuticaudata	5222	240.73	114.88	171	0
		Eclectus roratus	6700	701.68	221.1	480.76	0
		Melopsittacus undulatus	1220	84.35	59.64	28.99	0
		Platycercus elegans	3822	348.05	158.3	123.86	0
		Polytelis swainsonii	3149	288.77	170	153	0
		Psephotus haematonotus	1914	174.56	73.47	61.39	0
		Psittacula krameri	4239	565.67	120.45	116.11	0
		Trichoglossus haematodus	3726	409.77	125.6	113.25	0
Rheiformes	Rheidae	Rhea americana	19228	2295.13	1286.6	23000	1
Sphenisciformes	Spheniscidae	Spheniscus magellanicus	16757	2362.55	672.29	4105.1	0
Strigiformes	Strigidae	Aegolius acadicus	2857	743.75	64.49	100.69	1
		Athene cunicularia	5878	1707.67	148.71	150.61	1
		Ninox boobook	5626	1503.68	148.15	282.19	1
	Tytonidae	Tyto alba	6149	1605.41	136.51	403.32	1

7.3 Results

7.3.1 Brain shape descriptions of *C. pectoralis* and *C. vexillarius*

The outline of the brain of both species, which were distinguishable through the nearly transparent braincase (Figure 7-3A-B), established that both brains had shrunken over time as they were not occupying 90% of their braincase (Zusi, 1993; Iwaniuk and Nelson, 2002; Jerrison, 2012). The orbit of both nightjar species appeared to roughly occupied 45-50% of the skull. The eye sockets were separated by a thick interorbital septum, therefore the orbits sat apart, on the side of the skull, but angled so that they converged towards the front of the head (Figure 7-3A-B).

The overall brain size of *C. pectoralis* was slightly smaller than *C. vexillarius*; *C. pectoralis* had a brain length of 14 mm, a width of 12 mm, whereas *C. vexillarius* had a brain length of 15 mm, a width of 13 mm. The brain of both species had clear, functionally distinct brain regions, as it would be expected from any vertebrate brain. Both species had a similar external brain morphology, with the telencephalon lobes, Wulst, cerebellum, optic lobes and mesencephalon being clearly visible (Figure 7-3C).

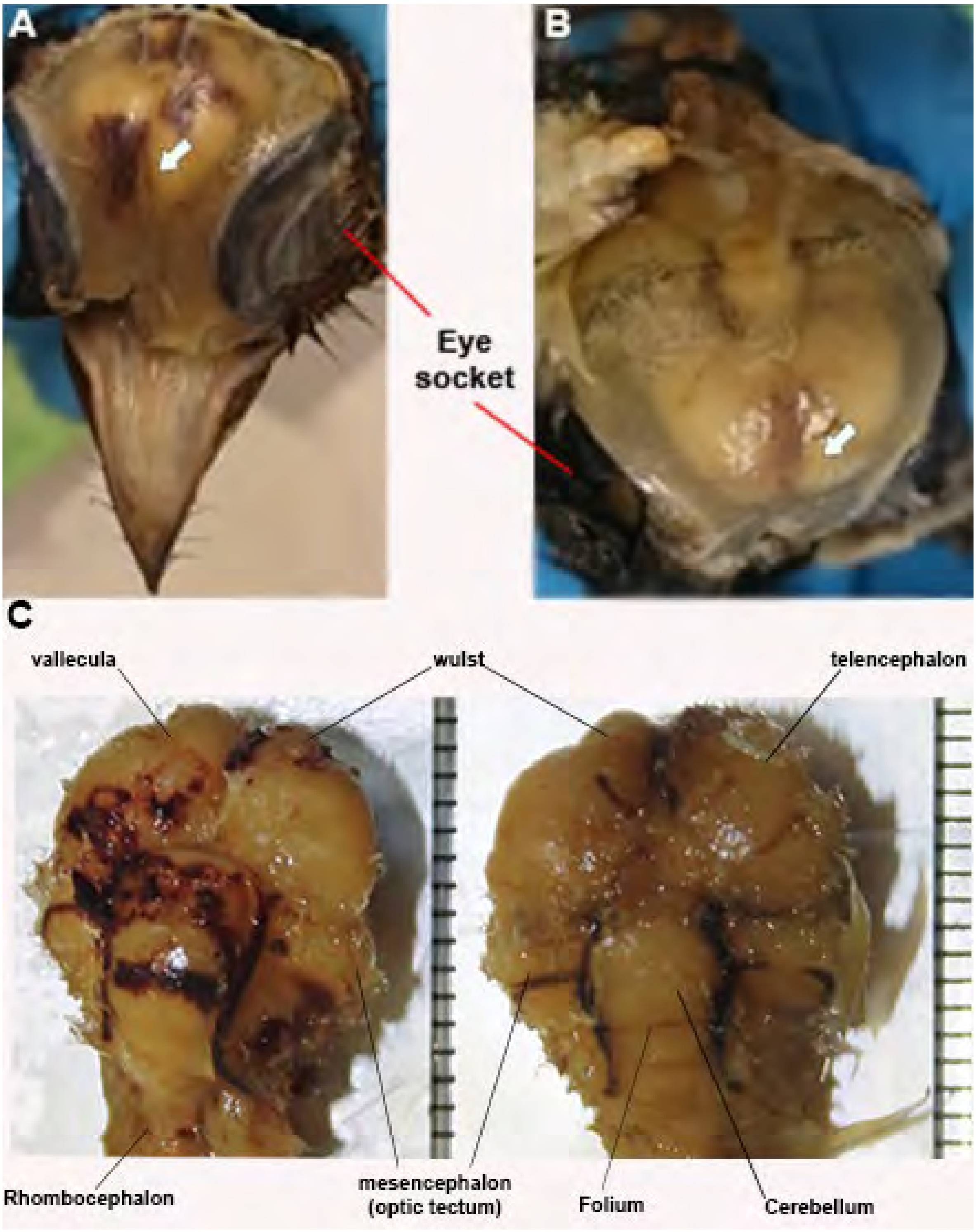


Figure 7-3. **Photos illustrating the position of the brain in their braincase** for **A)** *Caprimulgus pectoralis* and **B)** *Caprimulgus vexillarius*. **C)** Different areas of the brain of *C. vexillarius* (right) and *C.* pectoralis (left) labelled. The white arrow illustrates the position of the Wulst.

7.3.2 Brain anatomy of *C. pectoralis*

Since the brain specimen of *Caprimulgus vexillarius* was slightly perished, it was not possible to provide a full quantitative description of its neuroanatomy. However, both species revealed clear structures that have previously been associated with sensing in avian species (Figure 7- 5-7; Chapter 2). These will be described here from the spinal chorda via the mesencephalic tract to the telencephalon; particularly focussing on the rostral Wulst.

The spinothalamic tract passes through the mesencephalon; first, posteriorly through the midbrain (top sections in Figure 7-4), then anteriorly through the thalamus (bottom sections in Figure 7-4). The series of coronal sections through the optic lobe displayed in Figure 7-4, illustrated the laminar cytoarchitecture of the avian optic tectum (TeO). The optic tectum of *C. pectoralis* contained 15 layers agreeing with Ramon y Cajal's (1911) layering scheme Figure 7-5); however, there was some variation in the layers compared to that of the pigeon (*Columbia livia*) (Wylie *et al.* 2009). For example, layer 13 was clearly larger than that of the pigeon (Figure 7-5C), and was identifiable in the contralateral tectum in the midbrain, by dark staining of many cells (arrows in Figure 7-4).

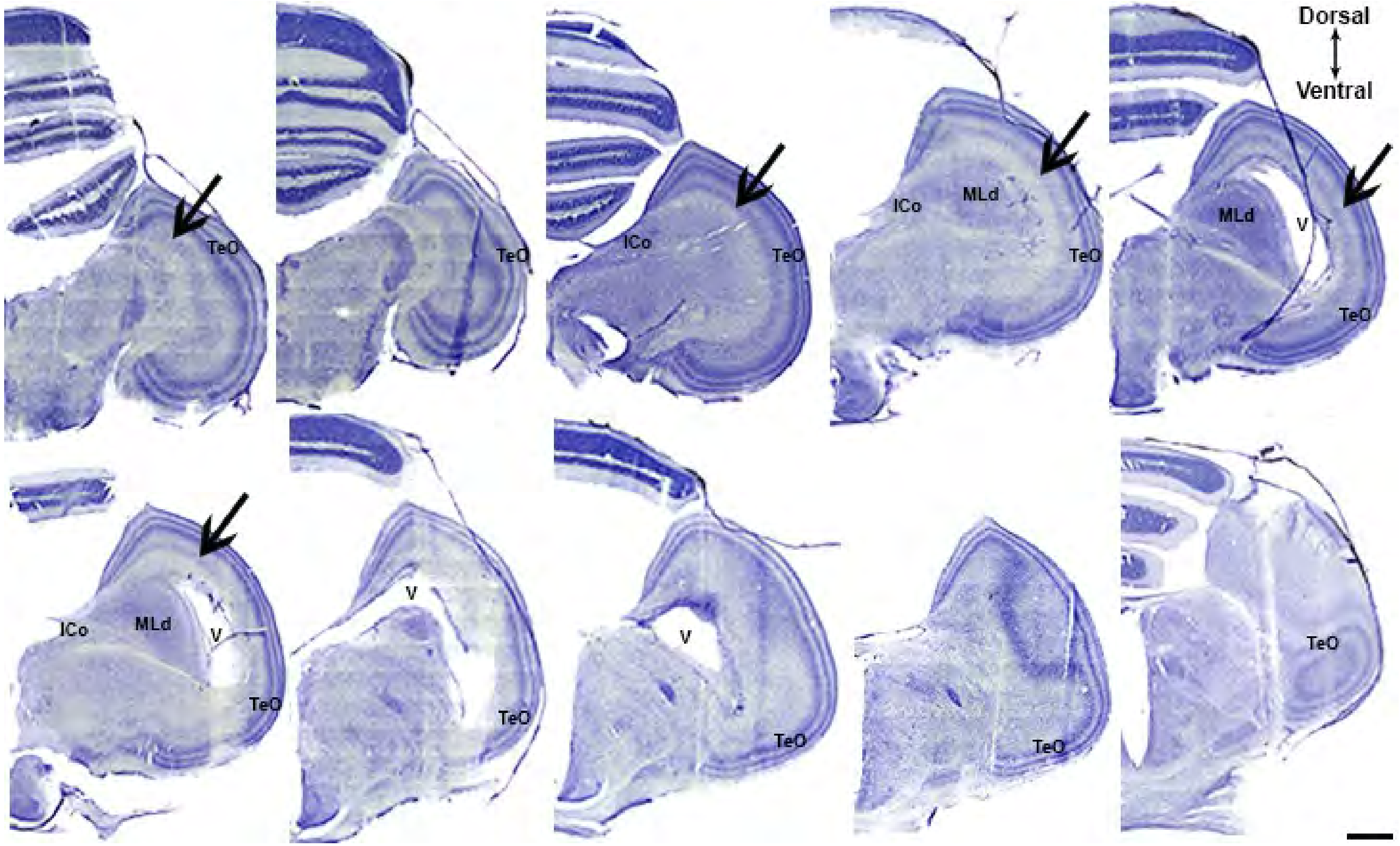


Figure 7-4. **Series of coronal Nissl-stained half-sections through the mesencephalon of *Caprimulgus pectoralis* brain illustrating the optic tectum**. Sections start posteriorly (top left corner) in the midbrain and extend anteriorly to the thalamus (bottom right corner). The optic lobe comprises the optic tectum (TeO), nucleus intercollicularis (ICo), the nucleus mesencephalicus lateralis, pars dorsalis (MLd) and the ventriculus lateralis (V). Scale bar = 1.000µm. Arrows pinpoint layer 13 of the optic tectum when visible.* notify which section was chosen in Figure 7-6 to present elements through which the sensory pathways project in to.

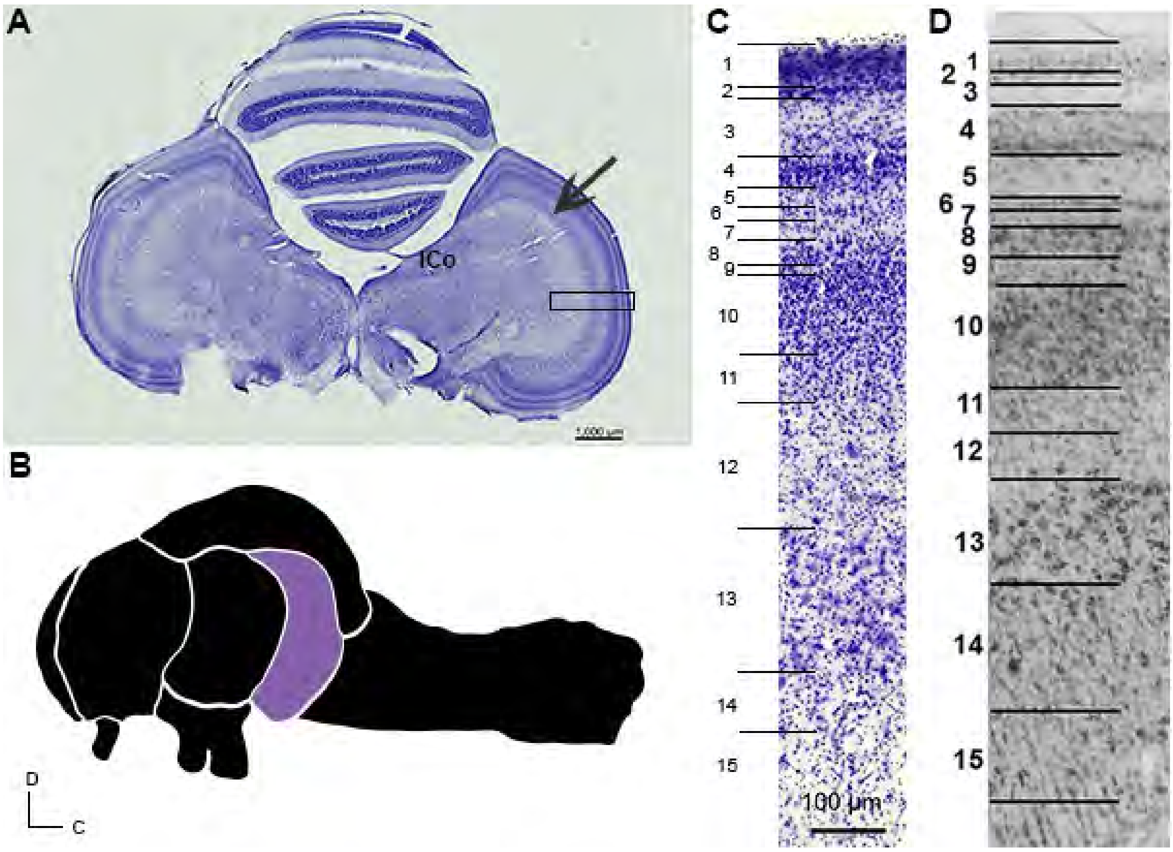


Figure 7-5. **A) Nissl stained coronal section of *Caprimulgus pectoralis* midbrain, illustrating the optic tectum laminated cytoarchitecture**. The rectangle depicts the region of interest taken for the photomicrograph of the optic tectum layers and the arrow pinpoint layer 13. **B**) Schematic lateral view of the *Caprimulgus pectoralis* brain, showing the midbrain (purple) from which the aforementioned coronal section is from. **C)** Photomicrograph of the optic tectum of *C.* pectoralis showing the different layers identified according to Ramon y Cajal (1911). **D)** Photomicrograph presenting the 15 layers of the optic tectum in *Columbia livia* following Ramon y Cajal (1911)'s same schema (figure modified from figure 2 in Wylie *et al.* (2009)).

Visual and somatosensory pathways project from layer 13 of the optic tectum to the thalamus, especially the nucleus rotundus (Rt) of the ipsilateral diencephalon (Figure 7-6). A few other thalamic landmarks were distinguishable in Figure 7-6, including the nucleus ovoidalis (Ov, associated with auditory pathways), the ventral lateral geniculate nucleus (GLv, two nuclei receiving projections from layer 13 and 9-10 of the optic tectum, respectively) and the principal sensory trigeminal nucleus (PrV), where trigeminal beak afferents are likely to project to (Gutiérrez-Ibáñez et al., 2009; Iwaniuk and Wylie, 2020). The nucleus rotundus was larger than GLv and PrV in this section (Figure 7-6). The dorsalis intermedius ventralis anterior (DIVA) in the dorsal thalamus that projects to the rostral Wulst was not distinguishable (Figure 7-5, 6).

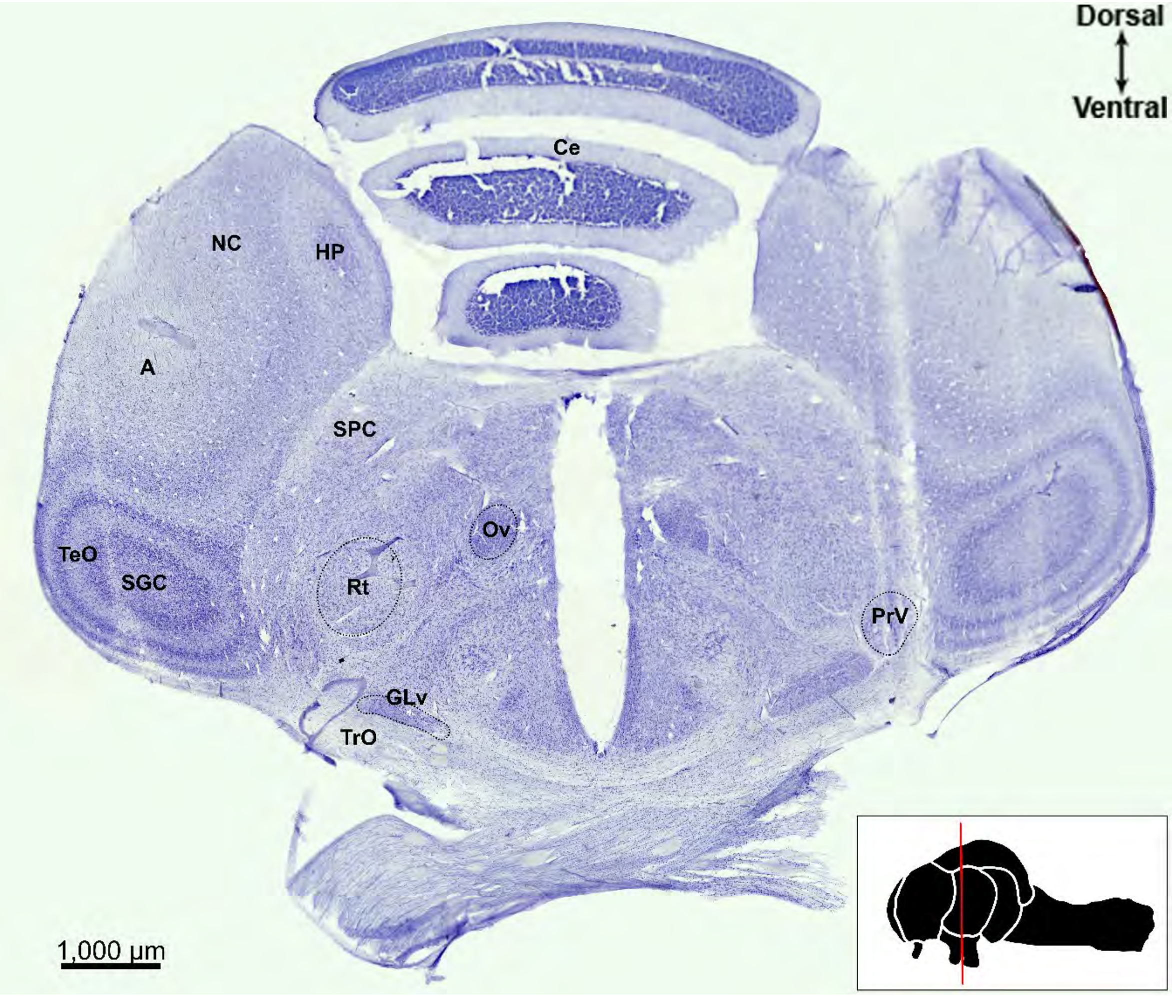
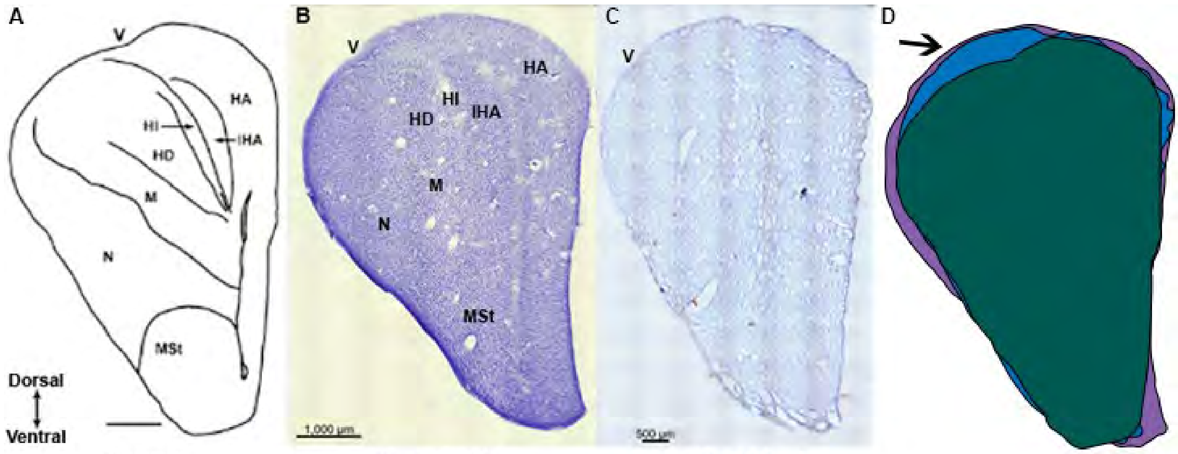


Figure 7-6. Nissl-stained section of the thalamus of *Caprimulgus pectoralis*, where the mesencephalon (optic tectum, TeO) **starts anteriorly** (cf. red line in the bottom right corner schematic). The section showed different elements involved in sensory function. A: archistriatum, GLv: nucleus geniculatus lateralis ventralis, HP: hippocampus, NC: neostriatum caudal, Ov: nucleus ovalis, PrV: nucleus sensorius principalis nervi trigemini, Rt: nucleus rotundus, SGC: striatum griseum centrale, SPC: nucleus superficialis parvocellularis, and TrO: tractus opticus.

Sensory projections from the thalamus (especially from the PrV and Rt) enter the telencephalon - into the nucleus basorostralis (Bas) in the rostrobasal part of the telencephalon and the Wulst, respectively. Although Bas was not clearly distinguishable in the *C. pectoralis* brain slices, the laminated cytoarchitecture of the Wulst was clearly apparent (Figure 7-7). The laminated cytoarchitecture of the Wulst region, along with its overall shape, was variable between species, even within the Caprimulgiformes. The contour of the Wulst was more rounded and wider in *C. pectoralis*, especially dorsally (purple outline, arrows in Figure 7-7D), compared to *C. vexillarius* and *E. argus*; *C. vexillarius* also had a rounder and bigger dorsal Wulst than *E. argus* (blue outline, arrow, Figure 7-7D).

The poor quality of the *C. vexillarius* tissue did not permit the identification of any of the Wulst regions; however, variation could be observed between *C. pectoralis* and *E. argus*. In *C. pectoralis*, the marking in the pallium, - constituting of the upper part of the section extending from the nidopallium (N) to the hyperpallium apicale (HA)- appeared to start laterally and more dorsally to the delineation of the vallecula compared to *E. argus*. These markings differed with the position of the outline between the hyperpallium intercalatum (HI) and where the interstitial nucleus of the apical hyperpallium (IHA) finishes. In *C. pectoralis*, the outline tended to go down into the sub-pallium – the part underneath the nidopallium – and meet the line delineating the medial striatum (MSt) Figure 7-7B). However, in *E. argus*, the equivalent outline tended to go no further than the mesopallium (M) and remained within the pallium (Figure 7-7A). Furthermore, the IHA region, which relays somatosensory inputs from the thalamus to HA, seemed to be wider in *C. pectoralis* than *E. argus,* resulting in a narrower HA. Likewise, HI was wider in *C. pectoralis*, with no apparent outline in the mesopallium to delineate it, as is the case in *E. argus*. Although the quality of *C. vexillarius* tissue did not allow a clear identification of the hyperpallium region, the position of the vallecular, i.e. furrow delimiting the Wulst region from the rest of the telencephalon, seemed relatively similar to *C. pectoralis*, and the dorsal section (where the hyperpallium apicale (HA) is) appeared to form the same wide rounded shape going from the delineation of vallecula to the inner lateral part of the section Figure 7-7B and C). *E. argus*, however, seemed to have a shorter and protruded dorsal section in between the vallecula delineation and the inner lateral part of the section (Figure 7-7A). The similarity in the contour of the dorsal section of the Wulst between *C. pectoralis* and *C. vexillarius* suggests that *C. vexillarius* may also have an HA that is more compressed dorsally and laterally than *E. argus*. If so, the anatomical regions of the *C. vexillarius* Wulst might also be more similar to *C. pectoralis* than *E. argus.*



E. argus *C. pectoralis* *C. vexillarius*

Figure 7-7. **Rostral sections of the telencephalon, showing particularly the Wulst in across three species**: ***Eurostopodus argus*** (A)*,* ***Caprimulgus pectoralis*** (B) **and** ***Caprimulgus vexillarius*** (C). **A)** Schematic drawing of the Wulst structure in *Eurostopodus argus* from Iwaniuk *et al.* 2006 (scale bar =1mm). **B)** Nissl stained section of the rostral part of *Caprimulgus pectoralis*' brain, showing the labelled Wulst (or hyperpallium) and other neighbouring regions. The dotted lines illustrate the markings outlining the different regions of the hyperpallium. V: vallecula, HA: hyperpallium apical, HI: hyperpallium intercalatum, IHA: interstitial nucleus of the apical hyperpallium, HD: hyperpallium densocellulare, M: mesopallium, N: nidopallium, Mst: medial striatum. **C)** Nissl stained section of the rostral part of *Caprimulgus vexillarius'* brain. **D)** Over-lying Wulst section of the three species: C. pectoralis (purple shape), C. vexillarius (blue shape), *E. argus* (green shape), which illustrates the difference in overall Wulst outline shape across species. The arrow pinpoints the dorsal part of the Wulst that is rounder in *C.* pectoralis than in *E. argus* and in *C. vexillarius*.

7.3.3 Wulst morphology

Comparison between C. pectoralis and C. vexillarius

The Wulst region was smaller in *C. pectoralis* than *C. vexillarius* (Figure 7-8B and C) (except for the lateral right side of the Wulst indicated by an asterisk in Figure 7-8A). The anterior view of the *C. vexillarius* brain revealed a longer Wulst compared to *C. pectoralis* (Figure 7-8). In the lateral views, the rostral pole of the Wulst in *C. pectoralis* appeared to be immediately dorsal to the olfactory lobes, while in *C. vexillarius*, it was positioned more dorsally to the olfactory lobes (arrows in Figure 7-8). The whole Wulst of *C. vexillarius* appeared to sit more dorsally on the telencephalon than that of *C. pectoralis*.

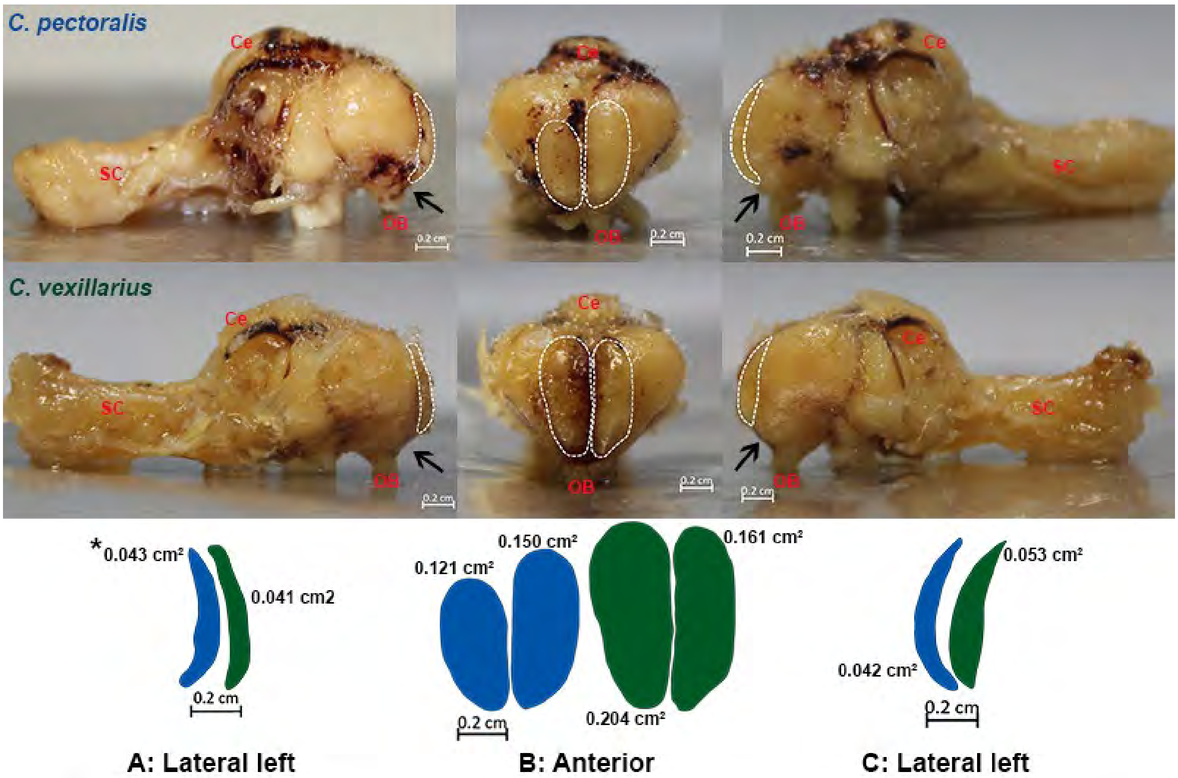


Figure 7-8. **Photos of *Caprimulgus pectoralis* and *C. vexillarius'* brain under three different views**: **A)** lateral right; **B)** anterior; **C)** lateral left. For both species, photos illustrate the outline of the Wulst for each view (dotted lines), where the Wulst shape was extracted and presented underneath the photos for each view. The asterisk indicates the only shape of the Wulst that is bigger in *C. pectoralis* than in *C. vexillarius*. Scale bar = 0.2 cm. Different areas of the brain were labelled in red: Olfactory lobes (OB), Cerebellum (Ce), Spinal chorda (SC). Arrows pinpoint the gap area in between the Wulst and the olfactory lobe.

Comparison of C. pectoralis and C. vexillarius with other nocturnal species

Brain shape varied across species, although some commonalities were observed. In all species, the telencephalon was larger than the other brain regions. Its lobes did not envelop the lateral aspect of the cerebellum in *E. argus, P.strigoides, C. pectoralis, S. caripensis* and *C. vexillarius*. In *N. boobook, T. alba* and *A. insignis*, the lateral aspect of the cerebellum was less exposed, covered by part of the telencephalon hemisphere (Figure 7-9). The size and position of the mesencephalon varied across the species. It was large, in the same sagittal alignment and at about the horizontal plane dorsally as the telencephalon in *E. argus, P. strigoides, C. pectoralis* and *C. vexillarius;* although *P. strigoides* mesencephalon was positioned more ventrally than the mesencephalon of other aforementioned species. It was smaller and positioned in an almost parallel plane position to the ventral surface of the telencephalon in *T. alba, N. boobook, S. caripensis,* and *A. insignis*. The olfactory lobes size, shape and position also varied between species (Figure 7-9). They were prominent in *E.*

argus, C. pectoralis, N. boobook, A. insignis, C. vexillarius, and especially in *S. caripensis*; they were not noticeable in *P. strigoides* and *T. alba* lateral view (Figure 7-9).

The Wulst shape, size and position also varied between species (Figure 7-9). Wulst shape appeared to be more similar in species from within the caprimulgiform order, including *E. argus, P. strigoides, C. pectoralis, S. caripensis, A. insignis* and *C. vexillarius*. *T. alba* and *N. boobook* had a similar crescent shape to the Caprimulgiformes, although their Wulst was bigger. *S. caripensis* of the Caprimulgiformes had a Wulst shape closer to *N. boobook*. In all species, the Wulst had a half-moon/crescent shape that was more enlarged rostrally, compared to the caudal part, which seemed more extended dorsally and ventrally (Figure 7-9). Similar to *C. vexillarius*, the Wulsts of *S. caripensis* and *E. argus* were positioned more dorsally than *C. pectoralis* (Figure 7-9). The Wulsts of *P. strigoides, T. alba, N. boobook* and *A. insignis* were all of different sizes and shapes, and extended more dorsally on the telencephalon and ventrally close to the olfactory lobes (Figure 7-9).

N. boobook had the largest Wulst area (0.691 cm^2), followed by *T. alba* (0.561 cm^2), which were about twice the size of the *S. caripensis*, *P. strigoides* and *A. insignis* (0.301 cm^2, 0.244cm^2 and 0.312cm^2 respectively; Figure 7-9). *E. argus, C. pectoralis* and *C. vexillarius* had the smallest Wulst areas (0.036, 0.043 and 0.041 cm^2 respectively; Figure 7-9). *E. argus* has the smallest Wulst volume (0.0604), then *Caprimulgus sp.* (0.0703), *S. caripensis* (0.1922), *P. strigoides* (0.2310), *A. insignis* (0.2361), *N. boobook* (0.2721), *T. alba* (0.2611). Wulst volumes represented around 40 % of the entire telencephalon volume in *N. Boobook* and *T. alba*, about 32 % in *P. strigoides* and the *A. insignis*, 26 % in the *S. caripensis* and less than 16% in *E. argus, Caprimulgus sp.* (Table 7-2).

There was no correlation between Wulst volume with bristle length (S = 14, rho=0.3, *p*=0.68), the number of mechanoreceptors at rictal bristle follicle (S = 38, rho=-0.09, *p*=0.86), nor bristle number (S = 6, rho=0.4, *p*=0.75). Species with large numbers of mechanoreceptors did not have bigger Wulsts, i.e. *E. argus* and *S. caripensis* that have 10 Herbst corpuscles around their rictal bristle follicles and had rather small Wulst volumes (Table 7-2).

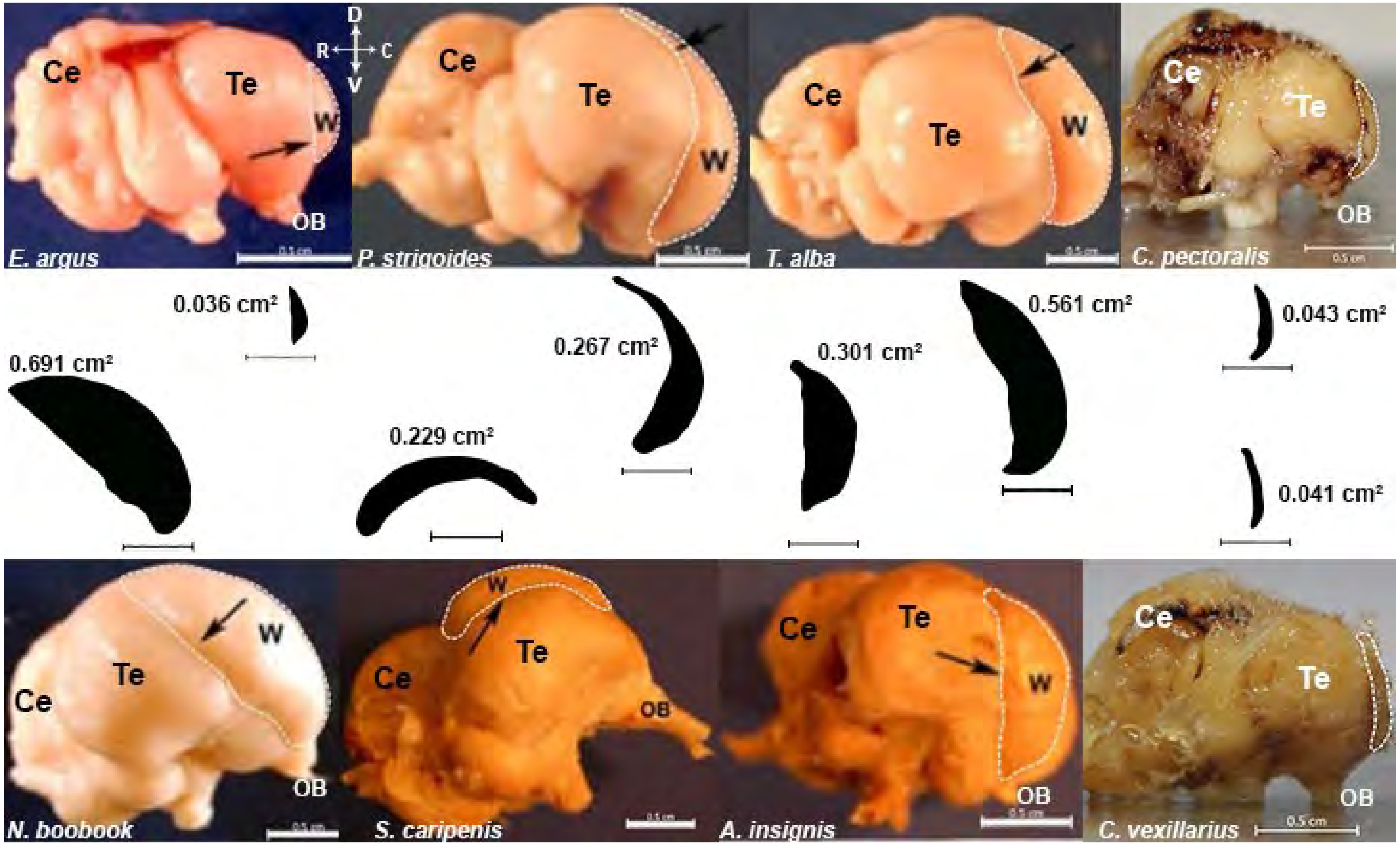


Figure 7-9. **Lateral view of the 6 avian species present in Iwaniuk and Wylie, 2006** (*Eurostopodus argus, Podargus strigoides, Tyto alba, Ninox boobook, Steatornis caripensis and Aegotheles insignis*), **plus *Caprimulgus pectoralis* and *C. vexillarius***. The photos show the delimitation of the Wulst, of which the shape has been extracted and presented in the middle, for each species (scale bar= 0.5cm). Ce: cerebellum, OB: olfactory lobes, Te: telencephalon, W: Wulst, and the black arrows pinpoint the vallecula. Rostro (D)-caudal (C) arrow, dorso (D) – ventral (V) arrow.

Table 7-2. **Summary table of the different measurements of the brain** (yellow heading) **and rictal bristles** (red heading, cf. chapter 3 and 4). Measurements were taken from Iwaniuk and Wylie (2006): Brain volume, telencephalon volume, Wulst volume. Wulst volume controlled is the Wulst volume controlled by the brain volume. Number of mechanoreceptors (i.e. Herbst corpuscles), rictal bristle length and number measurements come from Chapter 3. Ecological traits (Billerman *et al.*, 2020) that are possibly associated with the previous measurements were added as well. * mean of both lateral Wulst areas measured for *C. pectoralis* and *C. vexillarius*.

Species	Brain volume (mm^3)	Telencephalon Volume (mm^3)	Wulst volume (mm^3)	Wulst volume controlled	Wulst volume % of Telencephalon	Lateral Wulst area (cm^2)	Number of mechano-receptors	Bristle length	Bristle number	Foraging habitat	Foraging method	Period of activity
E. argus	982	426.73	59.30	0.0604	13.90	0.036	10	9.58 ± 1.23	16 ± 2	Semi-open	Hawking, Sallying	Crepuscular Nocturnal
P. strigoides	5311	3826.81	1226.89	0.2310	32.06	0.267	4	29.15 ± 2.24	20.8 ± 1.5	Closed	Gleaning	Crepuscular Nocturnal
T. alba	6,149	4108.53	1605.41	0.2611	39.08	0.561	7	12.56 ± 3.39	NA	Open	Sallying	Nocturnal
N. boobook	5,526	3920.44	1503.68	0.2721	38.35	0.691	NA	NA	NA	Semi-open	Gleaning, Hawking	Crepuscular Nocturnal
S. caripensis	3,900	2887.70	749.53	0.1922	25.96	0.229	10	36.36 ± 7.84	15 ± 1.4	Closed	Gleaning	Crepuscular Nocturnal
A. insignis	1540	1107.99	363.63	0.2361	32.82	0.301	NA	NA	NA	Closed	Hawking, Sallying	Nocturnal (Crepuscular)
Caprimulgus sp.	734	342.75	51.62	0.0703	15.05	NA	NA	NA	NA	NA	NA	NA
C. pectoralis	1290	NA	NA	NA	NA	0.043*	10	20.55 ± 0.59	10 ± 0	Semi-open	Sallying	Crepuscular Nocturnal
C. vexillarius	1550	NA	NA	NA	NA	0.047*	0	15.83 ±3.67	6.5 ± 0.7	Semi-open	Hawking	Nocturnal cathemeral

7.3.4 Association of rictal bristle presence and brain volumes

Species with rictal bristles present could tend to have larger normalised Wulst volumes than species without rictal bristles (phylogenetic Anova, F=9.6, df=45, *p*=0.05; Figure 7-10), although this trend was not significative here. There was no difference in the overall normalised brain volume (phylogenetic Anova, F=0.07, df=45, *p*=0.86; Figure 7-10) and normalised optic tectum volume (phylogenetic Anova, F=1.44, df=45, *p*=0.48; Figure 7-10) between species with and without rictal bristles.

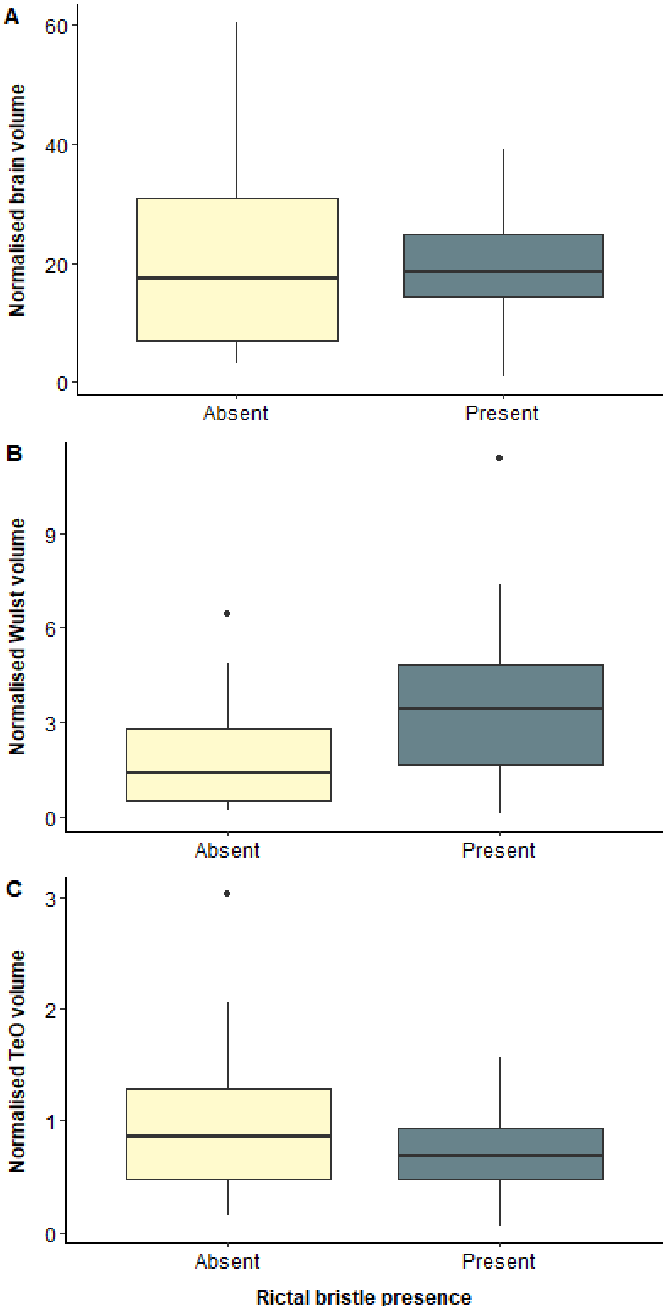


Figure 7-10. Boxplots of volume of specific brain areas against the rictal bristle presence/absence in 45 species from Iwaniuk *et al.* (2008)**,** illustrating the results of the phylogenetic anova of the rictal bristle presence with **A)** the normalised brain volume, **B)** the normalised Wulst volume and **C)** the normalised optic tectum (TeO) volume.

7.4 Discussion

Brain shape and anatomy

Integrating multiple senses is thought to have driven the increase in brain size and complexity in mammals (Rowe *et al.* 2005). If this was also the case in birds, species that are both tactile and visual (i.e. *Caprimulgus pectoralis*) may have larger brain sizes, with larger tactile and visual areas, than more visual species (i.e. *Caprimulgus vexillarius*); however, this was not the case. Overall, the gross brain shape and structure was relatively conserved between *Caprimulgus pectoralis* and *Caprimulgus* vexillarius, with *Caprimulgus pectoralis* even having a smaller brain than *Caprimulgus vexillarius*. The nightjars and oilbird have been found to have smaller brains than owls, frogmouths and the owlet-nightjar (Iwaniuk and Wylie, 2006). Brain size is complex, but is thought to be associated with life-history traits (Bennett and Harvey, 1985); for instance, larger brains are thought to be associated with sallying (hunting from perch to perch/ground) (Bennett and Harvey, 1985). *N. boobook, P. strigoides, A. insignis* and the nightjars, *C. pectoralis, C. vexillarius* and *E. argus* all sally (Table 7-2). However, *S. caripensis* forages by gleaning in closed habitat nocturnally and has a larger brain than both *C. vexillarius* that hawks in semi-open habitat diurnally and *Caprimulgus pectoralis* that sallies in semi-open habitat nocturnally (Table 5-1). The data from this study demonstrates that simple foraging preferences cannot account for the observed variation in brain size.

Sensory and cognitive abilities of a species, and possibly its ecology and behaviour, are all presumed to be associated with brain size and shape (Iwaniuk and Hurd, 2005). Regions such as the telencephalon, cerebellum and optic lobes of the mesencephalon are used to describe brain shape (Walsh and Milner, 2011). An increase in the size of these regions, relative to each other and the brain as a whole, is thought to be associated with an increase in neuronal density and, in turn, in the importance of the function carried by these regions (Striedter, 2005). Furthermore, changes in brain shape usually coincide with changes in the shape of the telencephalon, the cerebellum and the brain stem, which are regulated by the strength of their neuronal connection (Kawabe *et al.*, 2013). My results showed that all species, apart from the owls and the owlet-nightjar, have exposed cerebellums (Figure 7-9). Enlargement of the telencephalon tended to push the cerebellum posteriorly, inducing a ventro-rostral rotation of the brain stem (Kawabe *et al.*, 2013), which could explain the exposed the cerebellum in these species. Although, the cerebellar morphology differed within the five Caprimulgiform families, the owlet-nightjar, nightjars and potoos have a similarly-shaped and exposed cerebellum that differed from the owls and the frogmouth. The oilbird cerebellum shared features similar to the nightjars (Iwaniuk et al., 2005). This could suggest that species with exposed cerebellums, the owlet-nightjar and the owls, may have an expanded telencephalon, which has been associated with complex cognitive behaviours (Iwaniuk et al., 2005). Therefore, the owlet-nightjar and owls may have more developed cognitive capacities than the nightjars and frogmouths.

The optic lobes are primarily visual, but also contain structures associated with auditory (i.e. Torus (Cobb, 1964)) and mechanosensory (i.e. layer 13 or stratum griseum central of the optic tectum (Reiner and Karten, 1982) processing, which makes them especially interesting to look at here. Previously, visual specialists, like Charadriiformes have been thought to possess larger optic lobes than tactile feeding specialists such as Scolopacidae, Anatidae and Phoenicopteridae (Boire and Baron, 1994); although this was not so clear in my study. Owls (*T. alba, N. boobook*), the oilbird (*S. caripensis*) and owlet-nightjar (*A. insignis*) had smaller optic lobes than the nightjars (*C. pectoralis, C. vexillarius, E. argus*) and frogmouth (*P. strigoides*) (Figure 7-9). My data suggests that *S. caripensis*, *C. pectoralis* and *E. argus* are the most tactile species, with the most mechanoreceptors, but only *C. pectoralis* and *E. argus* have large optic lobes; therefore, there does not seem to be a clear association with tactile function and optic lobe size in the species studied here (Table 5-1). The visual and somatosensory layer 13 of the optic tectum also appeared to be larger in *C. pectoralis* with more neurons present than in pigeon. Since *C. pectoralis* is nocturnal with rictal bristles containing many mechanoreceptors (~10), this enlargement of layer 13 may be associated with nocturnal vision or an increased tactile function. Comparing this layer over more species will confirm this, especially exploring whether this enlargement is particular to the Caprimulgiformes or occurs widely in more tactile birds.

The somatosensory target, nucleus dorsalis intermedius ventralis anterior (DIVA) is the only nucleus projecting into the rostral Wulst (Funke, 1989), but was not visible in my sections. The nucleus basalis (Bas) and the principal sensory trigeminal nucleus (PrV) are also potential targets of tactile information; they receive information from the beak and these nuclei are especially enlarged in tactile foragers such as waterfowl, kiwis and probing shorebirds (Gutiérrez-Ibáñez et al., 2009; Cunningham et al., 2013; Iwaniuk and Wylie, 2020). Presumably, rictal bristle tactile inputs are also conveyed by the trigeminal nerve, however, nothing is known about their representation in the PrV or Bas (Iwaniuk and Wylie, 2020). Despite having many mechanoreceptors present in its mandible, around its rictal bristles follicles (Chapter 3; Delaunay *et al.*, 2020), the nucleus basalis of *C. pectoralis* was not identifiable and any enlargement of the PrV was not discernible (Figure 7-6). The quality of the tissue of *C. pectoralis* was not ideal, therefore no comparison could be made with *C. vexillarius*, which did not present mechanoreceptors at the follicles of its rictal bristles. Therefore, further investigation, such as integrative neuroanatomy coupled with behaviour, would be necessary to describe the representation of the rictal bristle in PrV, Bas as well as in other target brain areas.

Wulst morphology

The morphology of the Wulst varied across the species studied here, in terms of shape, position and volume. Iwaniuk and Wylie (2006) found that the morphology of Wulst was similar in owls (*N. boobook*) and frogmouth (*P. strigoides*), but dissimilar to the owlet-nightjar and the nightjar (*E. argus*). *C. pectoralis and C. vexillarius* revealed a similar Wulst shape to one another, but some differences with *E. argus*, especially in terms of shape. *E. argus* is a part of a clade of basal taxa, which is a sister group to Old World nightjars that include *C. pectoralis* and *C. vexillarius* (Barrowclough *et al.*, 2006; Han *et al.*, 2010), which might account for some of the differences. Overall, the Wulst organisation of these three nightjar species seemed more similar to one another than to the Wulst organisation of less related species, such as *A. insignis*, *P. strigoides* and *N. boobook* (described in Figure 5 in Iwaniuk and Wylie 2006); *N. boobook* Wulst organisation was perhaps the most dissimilar to the Caprimulgus species. These differences may reflect functional differences (Iwaniuk and Wylie, 2006), such as in the number of specific neurons (e.g. binocular neurons), or might be due to phylogenetic differences. From my data, I am not able to make this distinction.

The Wulst volume decreased from owls (*N. boobook* and *T. alba*) to owlet-nightjar (*A. insignis*) and frogmouth (*P. strigoides*). Wulst relative volume is thought to be associated with eye frontalisation and broad binocular overlap, and so possible global stereopsis (Martin et al., 2007; Iwaniuk et al., 2008). Therefore, it is not surprising that these species, with more forward-facing eyes, had larger Wulsts. Next was the oilbird with a moderately enlarged Wulst; however, despite a frontal eye position, no binocular neurons have been found in the oilbird Wulst (Pettigrew and Konishi, 1984). Therefore, it was suggested that the Wulst enlargement of the oilbird might be related to contour orientation (Nieder and Wagner, 1999, 2000), motion detection (Baron et al., 2007; Pinto and Baron, 2009), and somatosensory system inputs received in its rostral part (Iwaniuk and Wylie, 2006). The nightjars, *C. pectoralis, C. vexillarius,* and *E. argus*, had a smaller Wulst, which might reflect their narrower binocular visual field (25º; Martin *et al.*, 2004) and their more lateral eye position. Iwaniuk and Wylie (2006, 2020) found that nightjars and potoos (*Nyctibius griseus*) do not possess an enlarged Wulst. Conversely, waterfowl birds do have a large Wulst, which is likely to be due to somatosensory cues coming from their bill (Iwaniuk *et al.*, 2010). Despite somatosensory inputs having a likely effect on Wulst size and shape (Iwaniuk and Wylie, 2006), visual adaptations, at least qualitatively, appear to have more of an effect than rictal bristle somatosensory effects in my study. Indeed, Wulst volume was not correlated to bristle length, bristle number nor mechanoreceptor number in the species studied here.

Rictal bristles presence and brain anatomy

Rictal bristle presence might be associated with larger normalised Wulst volumes; however, no differences were found in the overall normalised brain volume and normalised optic tectum volume between species with and without rictal bristles. Of the 12 species with rictal bristles, only three were diurnal and the other nine were nocturnal, and could possess binocular vision. As larger Wulsts are thought to be associated with eye frontalisation and broad binocular overlap (Martin et al., 2007; Iwaniuk et al., 2008), the association between rictal bristle presence and Wulst volume could be due to nocturnal visual adaptations in the brain, rather than the rictal bristles themselves. However, despite having a relatively large Wulst, *S. caripensis*, is not thought to have binocular vision (Pettigrew and Konishi, 1984), and its visual field topography and retinal characteristics also indicate that *S. caripensis* do not rely upon visual cues to orient to food. Therefore, the enlargement of the Wulst in this species has been suggested to be mainly due to contour orientation (Nieder and Wagner, 1999, 2000), motion detection (Baron et al., 2007; Pinto and Baron, 2009), and somatosensory system inputs received in its rostral part (Iwaniuk and Wylie, 2006). *S. caripensis* possess long rictal bristles that have numerous mechanoreceptors around their follicles, suggesting that they have tactile sensitivity (Delaunay *et al.*, 2020) which could innervate the rostral part of the Wulst. Therefore, there may be an association between the Wulst volume and tactile rictal bristles could exist. If so, Wulst volume is likely to vary between species, and depend on both visual and tactile capabilities.

Sensing and brain anatomy

It is likely that any association between foraging traits, sensing and brain anatomy is complex; especially since brain structures, such as the trigeminal system, cerebellum, optic lobes and the Wulst receive information from many senses. Indeed, there are no clear relationships observed in any of the brain measurements, rictal bristle measurements and foraging traits studied here. Moreover, while the presence of rictal bristle in birds was found to be associated with larger Wulst here, this could be more the results of visual nocturnal adaptations than the presence of rictal bristle itself.

It is also likely that the species here are quite diverse in how they sense. As mentioned before, *S. caripensis* do not seem to rely entirely upon visual cues to orient to food, but might be complementing with using tactile cues from their rictal bristles at close range, and with echolocation and olfaction over longer distances Martin *et al.* (2004). According to behaviour evidence, visual cues are also not sufficient to explain nightjar nocturnal foraging (Salazar *et al.*, 2019). Salazar *et al.* (2019) proposed that their visual field helps in detecting prey above their head, while rictal bristles might locate prey coming from below (Salazar *et al.*, 2019). *T. alba* also possesses rictal bristles with mechanoreceptors at their follicle (Chapter 4), despite their large eyes and sensitive hearing used for prey localisation (Volman, 1994; Iwaniuk and Wylie, 2006).

Further investigations need to be conducted in avian sensing, especially investigating multisensory interactions and their associated brain areas. It is not yet clear how sensory signals from the rictal bristles are presented in the brain, although the trigeminal system, cerebellum, optic tectum and the Wulst are all interesting candidate structures. Brain anatomy varies considerably between species, and it is important to understand phylogenetic differences as well as functional adaptations. Future work should compare the neuroanatomy and sensory morphology of many species and conduct quantitative phylogenetic comparisons. This will be discussed more in the thesis Discussion chapter (Chapter 8, 1.2.2).

Conclusions

This is the first study to describe the neuroanatomy of *Caprimulgus pectoralis* and *Caprimulgus vexillarius*. *Caprimulgus pectoralis*, a more tactile species, had a smaller brain and Wulst size than the more visual, *Caprimulgus vexillarius*. I suggest that studying brain and Wulst sizes is complex and likely to be reliant on a number of factors. However, studying the optic tectum, especially in layer 13 might reveal some interesting tactile specialisations, as well as the thalamic nucleus DIVA and the telencephalic nuclei Bas and PrV. This study needs to be extended to include more species and include quantitative measures of neuronal density and connectivity, especially differentiating between visual and tactile Wulst areas. This will help us to better understand phylogenetic and functional neuroanatomical specialisations, especially in tactile species.

Chapter 8

Thesis discussion

Chapter summary

This final chapter consolidates the key findings, implications and limitations of this thesis, and makes recommendations for future work. As little is known about rictal bristles, I set out to characterise rictal bristle form and function in this thesis, by applying Niko Tinbergen's framework. In my thesis, I found that rictal bristle presence, morphology and follicle anatomy is diverse across birds. Some species have tactile rictal bristles, indicated by mechanoreceptors, Herbst corpuscles, around the bristle follicles. Tactile signals from the rictal bristles are likely to be processed by specialist brain areas, such as the Wulst, which is larger in species with rictal bristles. Rictal bristles are not present in young chicks, but emerge later and produce behavioural feeding responses when they are stimulated, which coincides with when chicks are independently feeding. The common ancestor of birds may have had rictal bristles. Rictal bristle presence is associated with nocturnality and some aspects of feeding. I suggest that rictal bristles play a role in foraging, collision avoidance and eye protection in species that forage in dark, complex environments. In order to investigate bristles function further, bristle use needs to be examined during natural foraging and feeding events in many species.

8.1 Introduction

In the introduction of this thesis (Chapter 1), I stated that facial sensors were found on many species - from invertebrate antennae, to vertebrate barbels, whiskers and bristles. I suggested that rictal bristles were an ideal candidate from which to further explore facial touch sensors in birds. However, little is known about these hair-like, fairly stiff and tapered bristles (Lederer, 1972; Pass, 1989). Therefore, I looked to mammalian whiskers for inspiration to investigate whether rictal bristles are also:

i) Associated with specialised follicle and neuroanatomical structures for touch
ii) Important in developing young
iii) Present in a common avian ancestor
iv) Associated with foraging in dark, complex environments

In order to assess these predictions, I applied Niko Tinbergen's framework (Tinbergen, 1963; Buchholz, 2007; Bateson and Laland, 2013). Hence, I set out to answer four questions: *How do rictal bristles work? How do rictal bristles develop? How did rictal bristles evolve?* And *What is the function of rictal bristles*? Answering these questions led me to explore rictal bristle morphology and anatomy, the development of rictal bristles, associated somatosensory brain areas, rictal bristle evolution, and finally the association of species-specific ecological traits, in order to characterise rictal bristle function.

8.2 How do rictal bristles work?

8.2.1 Rictal bristle morphology and follicle anatomy (Chapter 3)

As I predicted in Chapter 1, similar to mammalian whiskers, rictal bristle follicles of some avian species had Herbst corpuscles in their immediate surroundings, which are specialised structures related to mechanosensing. In particular, Herbst corpuscles are highly sensitive to pressure and rapid mechanical deformations such as vibrations (Gottschaldt, 1985; Necker, 2000; Stettenheim, 2000; Cunningham et al., 2013). The presence of these mechanoreceptors suggests that rictal bristles are capable of receiving tactile information in some species. However, contrarily to mammalian whiskers that have several types of sensory receptors at their follicle (Merkel endings, Pacinian and Ruffini corpuscles; Rice *et al.*, 1986; Muchlinski, 2010), only Herbst corpuscles were observed in the birds in our sample.

Variation in mechanoreceptor numbers was observed, which is consistent with my predictions from Chapter 2. In the Caprimulgiform species studied, *E. argus, C. pectoralis,* *A. cristatus* and *S. caripensis* had two times more mechanoreceptors present around their follicles than *P. strigoides, B. auritus, B. stellatus* and *C. europaeus*; whereas *N. albicollis. C. nacunda, C. minor* and *C. vexillarius* had no mechanoreceptors around their follicles. Just like in aquatic mammals that have more sensitive whiskers with 10x more nerve endings than terrestrial mammals (Hyvärinen and Katajisto, 1984; Hyvärinen, 1989; Marshall et al., 2006), Caprimulgiform species with twice as many mechanoreceptors could also have more sensitive rictal bristles than the other species.

The musculature of the rictal bristle follicles consisted of intrinsic muscle bundles connecting the follicle together, of which the size varied across species. Mammalian whisker musculature can also vary amongst species, such that diurnal species with less-organised whiskers tend to have disorganised and thinner intrinsic muscles (Muchlinski *et al.*, 2020). Mammalian intrinsic muscles give the ability of whiskers within the same row to move together (Dörfl, 1982). Although avian intrinsic muscles were rather small compare to mammalian intrinsic muscles (MRes Thesis: Delaunay, 2016; Figure 8-1), and do not form a sling around each follicle (Dörfl, 1982), these muscles may possibly enable rictal bristles to move forward and backwards as a group. Such sweeping movements have previously been observed in brown kiwis (*Apteryx mantelli*) (Cunningham et al., 2011). Therefore, like mammalian whiskers, rictal bristles could also be active touch sensors in some species.

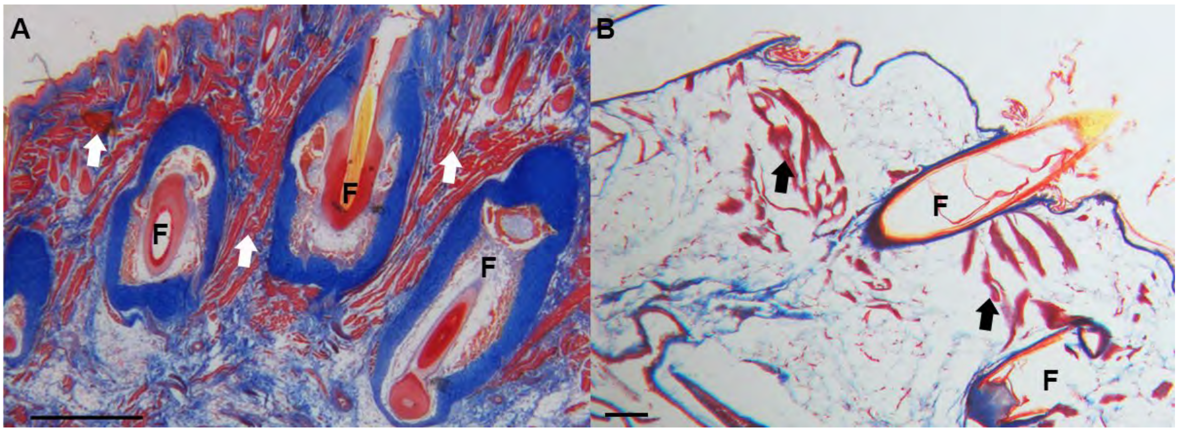


Figure 8-1. Follicle anatomy of A) whiskers of *Cavia porcellus* (guinea pig) and B) rictal bristle of *C. vexillarius,* illustrating the differences in musculature around the follicles (F) between mammals and birds. Both tissues were stained with Masson's trichrome staining. Arrows show the intrinsic muscle fibres. Scale bar in A =1mm. Scale bar in B=100μm.

I found rictal bristle morphology and follicle anatomy were diverse across the twelve Caprimulgiform species studied, with variation in the presence and number of mechanoreceptors, the follicle musculature, as well as bristle number, shape and length. In disagreement with my predictions in Chapter 2, there were no specific associations between mechanoreceptor number, rictal bristle morphology and foraging traits (e.g. nocturnality) observed in the Caprimulgiform species studied here. Although species with rictal bristles that lacked mechanoreceptors around their follicles tended to have short, thin and branched bristles and were associated with species that foraged pre-dusk and in open habitats.

8.2.2 Specialised somatosensory brain areas (Chapter 7)

In Chapter 1, I predicted that specialised somatosensory brain areas are likely to be associated with rictal bristles. In Chapter 2, I specifically predicted that species with mechanoreceptors around the rictal bristle follicles were likely to have a larger rostral Wulst as well as other associated mechanosensory brain areas, including the thalamic nuclei (i.e. Bas and PrV) and layer 13 of the optic tectum. I found that the optic tectum, and especially the associated somatosensory layer (layer 13), was larger in the nocturnal species, *C. pectoralis* than the nocturnal-cathemeral *C. vexillarius*. Layer 13 of the optic tectum is associated with both tactile and visual processing (Reiner and Karten, 1982). As *C. pectoralis* is one of the species with the most mechanoreceptors around its rictal bristle follicle, this suggests that the enlargement of layer 13 may be associated with the tactile function of the rictal bristles or with nocturnal vision. However, full quantitative measurements of layer 13, and other related mechanosensory targets, such as thalamic and telencephalic nuclei and PrV, as well as further species comparisons are needed.

The Wulst shape, position, volume and shape varied between species, with more similarities observed in more closely-related species. Therefore, Wulst differences might be due to phylogenetic relatedness as well as functional differences. *C. vexillariu*s had no mechanoreceptors around its rictal bristles follicles, but had a larger overall brain volume and Wulst volume than *C. pectoralis*, which had mechanoreceptors around its rictal bristle follicles. I compared the brain and Wulst volumes between the nocturnal species of Iwaniuk and Wylie (2006) (*E. argus, P. strigoides, S. caripensis, A. cristatus*, *T. alba*, *N. boobook*), which all had mechanoreceptors around their rictal bristles follicles (with the exception of *N. boobook* for which the follicle anatomy is not yet known). However, I found no clear associations of brain and Wulst volume with sensing (rictal bristle presence and number, and mechanoreceptor number) or foraging (activity period, foraging habitat and method). I went on to include more species (45 from Iwaniuk et al. 2008) from different orders to account for phylogenetic relatedness, and found that larger normalised Wulst volumes could potentially be associated with rictal bristle presence. However, there were no volume differences in the overall normalised brain volume and normalised optic tectum volume between species with and without rictal bristles. However, it should be considered that of the 12 species with rictal bristles, only three are diurnal and the nine others are nocturnal; therefore further species are needed to explore a potential association between rictal bristle presence, morphology and brain area volumes.

8.3 How do rictal bristles develop? (Chapter 4)

In disagreement with my prediction based on mammals made in Chapters 1 and 2, my results demonstrated that rictal bristles are unlikely to be functional in young chicks. The emergence of rictal bristles in *P. strigoides* and *T. alba* and their heightened behavioural responses coincide with independent feeding, suggesting that rictal bristles may play a role in feeding in older chicks and adults.

Mammal whiskers are present at birth, before eye-opening in both rats and mice (Grant *et al.*, 2012), suggesting the importance of whisker sensing in young mammals. Whiskers are thought to play an important role for newborn mammals, helping in nipple search and attachment, and huddling thermoregulation (Sullivan et al., 2003; Grant et al., 2012b). In birds, however, it seems that the tactile sensitivity of the rictal region develops gradually as the chicks get older, alongside the emergence of the rictal bristles and independence in feeding behaviour, which comes late in the development of the chicks. Indeed, both *P. strigoides* and *T. alba* are born without rictal bristles and their emergence started (~day 23 and 26, respectively) about two weeks after the eye-opening (~day 6 and 11, respectively), which suggests that they are not as important as mammalian whiskers in the early stages of development. Indeed, the rictal bristle developmental schedule and their function in chicks are very different from mammalian whisker development.

Although whisker development has not been studied in many species of mammals, it has been suggested to vary between species, depending on the degree of maturity of the young at birth (Grant and Goss, 2021). This could also be the case in birds. Indeed, although both altricial species, *P. strigoides* and *T. alba* had different development schedules, with *P. strigoides* appearing to be more mature at birth than *T. alba*, and their rictal bristles development also differed. Therefore, the development schedule of chicks, including feather development, rictal bristle emergence and eye-opening is species-specific, and likely to be dependent upon the level of parental care (precocial, super-precocial and altricial developmental types) and feeding behaviour.

8.4 How did rictal bristles evolve? (Chapter 5)

In agreement with my predictions in Chapter 1 and Chapter 2, rictal bristles are likely to have been present in an early ancestor of birds. Indeed, the phylogeny in this thesis went back to an avian ancestor 108mya, and rictal bristles were found in this most recent common ancestor (MRCA). Mammals are also thought to have a common ancestor with present and functional whiskers (Waite et al., 1998; Mitchinson et al., 2011; Grant et al., 2013a; Benoit et al., 2016). Other facial bristles, such as upper rictal, narial and interramal bristles were also likely to have been present in the MRCA, while lorial and lower rictal bristles were absent.

Feathers were present in theropod dinosaurs and have evolved in five different stages (Figure 2-2) (Prum and Brush, 2002; Gill, 2007), of which the stages I and II resemble rictal bristles. Indeed, stage I corresponds to an unbranched, hollow tubular feather and stage II corresponds to a feather with barbs attached to the calamus, resembling “semi-bristles”. This stage could correspond to the short rictal bristles with barbs at the base state retraced in the MRCA. Feathers developed their complex form over 108mya, with pennaceous flight feathers being present in *Protarchaeopteryx* (120-136mya). Therefore, rictal bristles might have been present sooner in the evolution of birds, acting either as a facial sensor or eye protection.

Unlike mammalian whiskers that are thought to have developed directly from early ectodermal and mesodermal mechanoreceptors (Maderson, 1972; Chernova, 2006), feathers are thought to have evolved as novel epidermal structures, developing from the selection of a feather follicle that originated from cylindrical epidermal invagination around the base of a dermal papilla, which would then grow a tubular appendage (Prum and Brush, 2002). Therefore, while whiskers, or “protovibrissae” in early mammals, were likely tactile, this might not be the case for the first apparition of feathers, or bristles, in birds.

Rictal bristle evolution underwent multiple events of gains and losses. Their presence and morphology are diverse between orders, families and genera, suggesting that phylogenetic relatedness is not the only driver of rictal bristle presence and morphology. Indeed, despite rictal bristle presence and morphology having a strong phylogenetic signal, they might also be influenced by life-history traits, such as those associated with foraging and nocturnal living. Indeed, as the MRCA of this study appeared to be diurnal cathemeral (80% confidence in ASR), and rictal bristles are particularly present in nocturnal extant species, I suggest that rictal bristles were mostly conserved in species that transitioned to nocturnality. Rictal bristle tactile sensitivity could then have been further enhanced in nocturnal species, by having longer and more sensitive bristles, and tactile sensitivity could have been reduced in more diurnal species. Longer rictal bristles are usually unbranched, rather than branched as the analysis demonstrated in Chapter 7. Perhaps longer, unbranched rictal bristles might provide more a larger sensory range, just like longer whiskers are found in small nocturnal, arboreal mammals, which are known to aid in object exploration and guiding locomotion (Grant et al., 2018).

8.5 What is the function of rictal bristles? (Chapter 6)

In confirmation with my predictions in Chapter 2, my results revealed that rictal bristle presence and normalised length were associated with species-specific ecological traits. However, these associations were complex and dependent upon a combination of life-history traits. Rictal bristle presence and length were associated with nocturnality, as well as some aspects of foraging, such as foraging method and diet. This supports my prediction made throughout the thesis that species foraging in low light conditions have tactile rictal bristles, while species foraging in lighter conditions and during daylight have a reduced tactile function.

Additionally, rictal bristles might have been lost and reduced in length in bird species that dive, hawk and glean, as well as those species that have a more flexible diet. This supports my prediction in Chapter 1 that birds foraging in complex habitats in the dark are more likely to have long rictal bristles. I also found that rictal bristles may be absent in species that forage at high-speeds - in open air or water. The disappearance of rictal bristles in these species might be to prevent damage, reduce drag and because selection for their retention is weak.

Furthermore, the foraging method scanning was associated with rictal bristle presence in many species. Therefore, the rictal bristles may play a role in guiding head orientation during scanning. Differences in head movement are related to the position of the eyes, their movement and their retinal structure, and may facilitate prey detection (O’Rourke *et al.*, 2010). *T. alba* does not detect prey moving sideways, possibly due to their low degree of eye movement and narrow lateral visual field (Shifferman and Eilam, 2004; O’Rourke et al., 2010). As this species was found with unbranched rictal bristles and mechanoreceptors around its bristle follicles, perhaps, rictal bristles aid in correcting that visual blind spot, by detecting vibrations caused by moving prey. In more visual species, which may be more likely to lack mechanoreceptors around their rictal bristle follicles, they may still be able to detect movement of the head via their rictal bristles, perhaps by using skin stretch sensors. Whiskers in mammals are also known to guide head orientation movements, and the whiskers will move to orient ahead of head rotations (Towal and Hartmann, 2006; Milne and Grant, 2014; Milne et al., 2020).

In conclusion, rictal bristle form and function are associated with species-specific life-history traits. Rictal bristles may act as facial sensors, but their exact role is likely to vary between species. Rictal bristles with Herbst corpuscles can detect vibrotactile stimuli. They may help prevent collisions in species foraging in low light conditions. In diurnal species, with few or absent mechanoreceptors, a reduced tactile function is expected. In these species, rictal bristles may act as proprioceptors during foraging and visual scanning. Rictal bristles are also likely to play a physical role in eye protection as birds move around complex habitats or feed on specific food items.

8.6 Limitations and future recommendations

Whilst this thesis has provided new insights on rictal bristle form, and established that rictal bristles function is complex and likely to vary between species, there are some limitations to this research.

8.6.1 Sample size quality of animal specimens

Follicle anatomy and neuroanatomy

The anatomy work was conducted on 12 species, of which only one specimen per species was dissected due to the availability of these species in the spirit collection at the National Museum of Tring in museums. Indeed, Caprimulgiform species are rare to find preserved in museums. However, a comparison of rictal bristles in as many as 12 species had never been done before, i.e. Küster, (1905) compared four owls while Cunningham *et al.* (2011) compared five New Zealand bird species. Nevertheless, further follicle anatomy studies should be conducted on more species, especially including diurnal and diurnal cathemeral species, which have not yet been investigated.

The quality of the tissue for histology varied between our specimens too. This is highly dependent on the time from the death of the animal to being put into preservative spirit. I had no control over this, and this timing is unknown for all specimens. Tissue degradation can modify the anatomical details found within the species studied. Long-term storage in spirit tends to be a primary cause of shrinkage in museum specimen soft-tissue (Hedrick *et al.*, 2018). Although formalin has been recognised to cause some degree of shrinkage in tissue (Ericsson and Biberfeld, 1967; Dam, 1979), formalin fixation only explain 1-5% of shrinkage when in concentrations lower than 40% (Fox et al., 1985; Baverstock et al., 2013; Düring et al., 2013; Hughes et al., 2016). Therefore, formalin alone does not explain the extreme size reduction in the soft tissue of in museum specimens (Hedrick *et al.*, 2018). Hedrick *et al.*, (2018). I was not able to access freshly collected specimens to assess if the tissue of preserved specimens reflected the anatomy of fresh tissue. Therefore, if one comes across freshly dead specimens it would be beneficial to assess the level of shrinkage between both types of tissue for rictal bristle anatomy, as well as to systematically assess the effects of formalin and other fixing and preservative agents on the tissue.

For the neuroanatomy work, I only had access to two specimens' brains, which was not sufficient to make clear conclusions. Problems with the quality of tissues were most notable in the neuroanatomy work, and quantitative measures of neuronal density and connectivity could not be conducted. However, museum spirit collections were the only way to access this type of tissue. Nevertheless, this was the first description of the brains of these two species and I was able to explore the regions of interest associated with processing mechanosensory signals. I recommend exploring these areas in more species and in more quantitative detail in the future, including the Wulst, layer 13 of the optic tectum and specific nuclei of the thalamus and telencephalon (i.e. DIVA, Bas, and PrV).

Developmental experiment

The developmental study was designed specifically for this thesis. Experimenters conducting the study in *P. strigoides* varied, as these were zookeepers that carried out the task. Therefore, the chicks were not always touched in the same sequence for the same number of times. Having a single operator performing the experiments over the development of the chicks would reduce disparities between the recorded videos. Having a specific time to record the video could also be beneficial, maybe ensuring that the chicks were not fed prior to the experiment so that they may be more responsive.

The set-up for *T. alba* was also constrained, as I could only start the experiment after the oldest chick was 13 days old, to reduce nest disturbance. Perhaps, for consistency and easy comparison with *P. strigoides*, this experiment should be carried out using captive chicks every day, to assess if the wild set-up had an influence on my results.

This was the first study to investigate behavioural responses to touch in birds. Data on *P. strigoides* development and nesting behaviour was lacking; therefore it was challenging to make direct comparisons with *T. alba*, which was more documented (Haresign and Moiseff, 1988; Durant and Handrich, 1998; Durant et al., 2008). Having more knowledge of the timing of *P. strigoides* developmental behaviour, such as independent feeding would be useful. Therefore, further work describing the development of chicks in *P. strigoides* is needed. Furthermore, the sensitivity of the rictal region to touch during the chick development and its association with feeding behaviour should be investigated further and include more species, especially comparing species that are diurnal and nocturnal, altricial and precocial. It would also be interesting to observing parent-offspring interactions during feeding, in order to determine whether the rictal bristles or rictal region is involved in soliciting behaviour and food delivery.

Rictal bristle presence and morphology

Taxidermied specimen from the museum skin collection were used to collect the presence and morphology data of rictal bristles. It is possible that older specimens could have had their rictal bristles plucked or damaged during taxidermy. I chose specimens in the best shape available to reduce such bias. Museum specimens can be more easily and accurately measured than live birds, especially for features, such as rictal bristles that can be very small Rosamond *et al.* (2020). These specimens enabled me to provide the first description of rictal bristles in a large sample of species. The collection of the presence of rictal bristles was sought to obtain a representative sample of avian species. According to Birds of the world numbers (Billerman *et al.*, 2020), my study represented ~10% of avian species, including 91 families (37% recorded families) and 29 orders (73% of all orders). Therefore, my data covers a wide range of different species and can still be thought of as representative of the avian phylogeny. While a sensitivity analysis was conducted to correct for potential sampling bias, it would still be useful to have a more balanced sample. For instance, 95% of the Passeriformes order, which is species-rich, were not sampled here, and as seen in Chapter 6, rictal bristle presence and morphology in this order was diverse. Therefore, sampling more Passeriformes species would be beneficial to this work. Additionally sampling more species of the Falconiformes, Otidiformes and Strigidae would be beneficial to this work too, as these were less sampled than other orders. Including more species for this work would also help reconstruct an older MRCA, and therefore help determine if rictal bristles were present in even older ancestors. The study of the evolution of rictal bristles could be extended further, as the genetic basis of rictal bristles could be explored in order to verify if different rictal bristle morphologies are homologous. An ancestral character state reconstruction analysis could also be conducted on the presence/absence of mechanoreceptors in species, to examine the appearance of tactile rictal bristles.

Studying the unlying genetic code of rictal bristles will also help us to confirm if different rictal bristle morphologies arise as the result of plasticity or as the results of evolutionary events, in which a phenotype is gain or lost. Therefore, studying rictal bristle morphologies and their associated genotypes should be considered for future work.

8.6.2 Ecological data

For the ecological data, I used the Cornell lab of ornithology, Birds of the World.org (Billerman *et al.*, 2020), which groups scholarly data available on one species. However, certain species that are more cryptic or less commonly studied had less ecological information collected and therefore less detailed data was available. These disparities in the level of ecological details available for each species might have caused species to be put in the wrong ecological categories for the analysis. Therefore, further work on collecting more ecological data should be done, especially the activity time and diet in rare species. My findings could change if more ecological details become available in the future, and the database Birds of the World (Billerman *et al.*, 2020) is constantly updated. Furthermore, nesting behaviour would also be interesting to investigate as it might have an influence on rictal bristles presence and function; however, due to the lack of data in many species, this parameter was not used in this thesis, but could be investigated in future work.

8.7 Remaining gaps of knowledge

This thesis is the first to describe the comparative form, development, evolution and function of avian rictal bristles. It forms the basis from which to further develop this research into avian rictal bristle sensing. Specifically, I recommend the following knowledge gaps still exist.

i) This thesis provides a comprehensive record of the presence/absence of rictal bristles and their form have been established but only covers ~10% recorded species, representing 418 genera, from 91 families (37% recorded families) and 29 orders (73% of all orders). This work also demonstrated that rictal bristle presence varied within species, families and order, and that short with barbs at the base rictal bristles were present in a MRCA, around 108 mya. Therefore, I suggest that this work should be extended to include more species, so the extent of the rictal bristle presence evolution can be observed, and an older MRCA can be reconstructed to determine the ancestral character state of rictal bristle presence and morphology.

ii) I found that rictal bristles are absent at birth and develop slowly in at least two altricial species (tawny frogmouth and barn owl); however, rictal bristle presence and development in diurnal altricial and precocial hatchlings are still unknown. I suggest that this should be looked at further, especially in barbets (Lybiidae, Megalaimidae, Capitonidae, and Semnornithidae), which are diurnal altricial species known to have rictal bristles present (an exact record of species with and without bristles could also be interesting to report), and the hoatzin, which is the only precocial species I recorded with rictal bristle present in the adult (Chapter 5).

iii) Throughout the thesis, I have found evidence that rictal bristle presence and form is associated with nocturnality, as well as some aspects of foraging. However, exactly how the bristles are used for orientation and foraging needs to be better understood, perhaps by conducting foraging experiments in the field.

iv) I propose that rictal bristles are likely to be tactile in more nocturnal species and could act as an early warning system to protect the eyes from vegetation or food items, but this needs to be further investigated for further confirmation. I recommend behavioural observations in the field and experimental observations in the lab might be able to explore this further.

v) Rictal bristles are likely to be associated with some mechanosensory associated brain regions, such as layer 13 or the Wulst. Full quantitative neuroanatomical measurements of layer 13, and other related mechanosensory targets, such as thalamic and telencephalic nuclei and PrV are needed. Exploring the neuronal pathways from the rictal bristles to the brain, perhaps by using electrophysiology or retrograde tracers would help to map these areas and pathways.

8.8 Ongoing further work

Developmental work

As demonstrated here, the developmental schedule of the chicks and rictal emergence differed between species, even if they are both altricial. Therefore, more species need to be investigated. During my PhD studies, as a side project, the developmental study has been extended to more species. I found that rictal bristles are also absent in European nightjar hatchlings (*Caprimulgus europaeus*; Figure 8-2A), however, no behavioural tests have been conducted yet.

Elora Grahame at the University of Guelph, Canada is currently carrying out my behavioural protocol in the field, during the development of common nighthawk (*Chordeiles minor*) and Eastern whip-poor-will (*Antrostomus vociferous*; Figure 8-2B) chicks. So far, data shows that these chicks appear to be more mature at birth than *P. strigoides* and *T. alba*. Although the behavioural data has not yet been analysed.

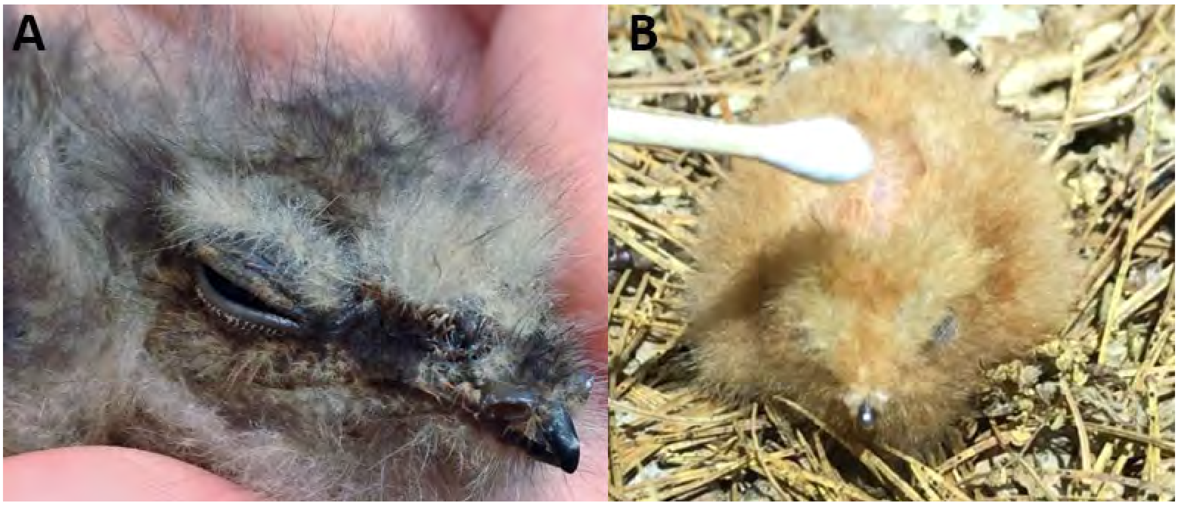


Figure 8-2. Photographs of a A) *C. europaeus* hatchling and B) *A. vociferous* hatchling. Tactility was conducted with a cotton bud in the wild on *P. nuttallii* by Enora Grahame.

Foraging behaviour

Investigating the foraging behaviour of species and observing their rictal bristle use while feeding could be useful to understand the use of rictal bristles during feeding. Indeed, as mentioned in section 8.2.1, rictal bristles may be moveable, therefore observing the feeding behaviour in species other than the Kiwis (Cunningham et al., 2011), could be beneficial to determine if rictal bristles actively move. Filming the feeding behaviour of nocturnal species with rictal bristles could help investigate further if rictal bristles are involved in prey capture. This has been trialled in the field in collaboration with Dr Lucy

Mitchell and Dr Kathryn Arnold from the University of York, as part of a study collecting data on *C. europaeus* ecology in the peatlands of Yorkshire, UK. I successfully collected some high-speed video footage (100 Fps) of adult *C. europaeus*; however, I was not able to see the rictal bristles in any footage (Figure 8-3). Having a more constrained filming arena, such as by using a moth trap, or conducting the experiments in captivity might be the way forward with this work.

Figure 8-3. Snapshot of a *C. europaeus* flying above the peatlands canopy, foraging for insects (Thorne, UK).

8.9 Wider implications and conclusions

By following Tinbergen's approach, I have been able to look at rictal bristles from different perspectives, including anatomical, developmental, neural, sensory and ecological. Tinbergen's framework provided me with a structure, which allowed me to better understand rictal bristle form and function. As so little was known about rictal bristles before my work, this research has considerably expanded our scientific knowledge of avian rictal bristle form and function.

Rictal bristles in some bird species act as facial touch sensors, indicated by the presence of mechanoreceptors around the bristle follicles. Facial touch sensors can be found across the animal kingdom as barbels in fish (Kasumyan, 2011), antennae in arthropods (Staudacher *et al.*, 2005) and whiskers in mammals (Muchlinski, 2010). Since rictal bristles are simple, tapered feathers contained within a follicle, they work in a similar way to whiskers. In species that have mechanoreceptors around the bristle follicles, vibrations of the bristles are translated by the mechanoreceptors, Herbst corpuscles, into neural signals, that are then processed by specialised mechanosensory brain areas, including the rostral Wulst (Delius and Bennetto, 1972; Karten et al., 1973; Pettigrew, 1979; Funke, 1989; Wild and Williams, 2000) and layer 13 of the optic tectum (Reiner and Karten, 1982). Species that do not have mechanosensors around the bristle follicles may still have functioning bristles; for example, stretch receptors in the skin may be able to detect bristle deformation. In addition, the physical structure of the bristle is likely to play a role in eye protection in all species.

Like barbels (Kapoor and Bhargava, 1967; Singh and Kapoor, 1967; Kasumyan, 2011) and whiskers (Dougill *et al.*, 2020), rictal bristles vary in number, morphology and anatomy between species, which is likely to be related to their life-history-traits. Species with rictal bristles tend to be nocturnal, and species that lack mechanoreceptors around their bristle follicles tend to be diurnal. Therefore, rictal bristles are probably associated with nocturnality and used in the dark, much like fish barbels underwater, and mammalian whiskers in nocturnal and aquatic species.

Unlike whiskers, rictal bristles are absent at birth and their emergence and behavioural responses coincide with independent feeding. Therefore, rictal bristles are likely to be associated with feeding or foraging. This is also true of mammalian whiskers, that guide orientation to moving prey, and localise attacks, especially in Australian water rats (*Hydromys chrysogaster*) (Dehnhardt et al., 1999), rock hyrax (*Procavia capensis*) (Sarko *et al.*, 2015) and etruscan shrews (*Suncus etruscus*) (Anjum *et al.*, 2006).

Rictal bristles were likely to have evolved in early birds, much like whiskers did in early mammals. I suggest that rictal bristles play a role in foraging, collision avoidance and eye protection in birds, especially in dark, complex habitats. The rictal bristles of the relatives of birds may have used their bristles in a similar way. Therefore, rictal bristles are facial sensors associated with touch, and give birds the ability to perceive vital information from their surroundings.

Contributions

Dr Robyn A. Grant and I (Mariane Delaunay) designed the structure and studies of this thesis. Dr Carl Larsen helped getting access to the spirit collection of the Natural History museum of Tring and the skin collection of the World museum of Liverpool. I carried out the majority of the data collection and performed all the analyses and interpretations. I made all the figures in the thesis. The thesis was critically reviewed by Dr Robyn Grant, Dr Carl Larsen, Dr Huw Lloyd, Dr Matthew Sullivan and Dr Charlotte Brassey.

Chapter 3 and 5: I performed all dissections, histology and statistical analysis. I also took all microscopic pictures.

Chapter 4: I designed the protocol and ethogram for the behaviour videos, which I sent to Mark Myers at the Woodland Park Zoo in Seattle to obtain frogmouth data. All videos of frogmouth chicks were taken by the staff at Seattle Zoo under my guidance, and sent over to me for analysis. The videos of barn owls were filmed both by myself, as well as by a student who accompanied me to Israel. All videos analysis and statistical analysis were done by me, except for the reliability analysis, which used a subset of scores from another PhD student.

Chapter 6 and 7: Biometric measurements were, in the majority, collected by myself alone, however I was sometimes helped in collecting the measurements by a student or R.G under my supervision. I executed all data modelling, statistical analysis and figures in R.

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Anatomy of avian rictal bristles in Caprimulgiformes reveals reduced tactile function in open-habitat, partially diurnal foraging species

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Abstract

Avian rictal bristles are present in many species of birds, especially in nocturnal species. Rictal bristles occur along the upper beak and are morphologically similar to mammalian whiskers. Mammalian whiskers are important tactile sensors, guiding locomotion, foraging and social interactions, and have a well-characterised anatomy. However, it is not yet known whether avian rictal bristles have a sensory function, and their morphology, anatomy and function have also not been described in many species. Our study compares bristle morphology, follicle anatomy and their association with foraging traits, across 12 Caprimulgiform species. Rictal bristle morphology and follicle anatomy were diverse across the 12 species. Nine of the 12 species had mechanoreceptors around their bristle follicles; however, there was large variation in their musculature, mechanoreceptor numbers and bristle morphology. Overall, species with short, thin, branching bristles that lacked mechanoreceptors tended to forage pre-dusk in open habitats, whereas species with mechanoreceptors around their bristle follicle tended to forage at night and in more closed habitats. We suggest that rictal bristles are likely to be tactile in many species and may aid in navigation, foraging and collision avoidance; however, identifying rictal bristle function is challenging and demands further investigation in many species.

KEYWORDS
feather morphology, feeding ecology, mechanoreceptors, nightjars, sensing

1 | INTRODUCTION

Across the animal kingdom, the sense of touch is one of the most specialised senses (Kidd, 1907), yet it remains one of the least studied and understood (Prescott and Dürr, 2015). This is especially true in birds, despite birds having touch-sensitive beaks (Gerritsen and Meiboom, 1985; Schneider *et al.*, 2014), and their feathers also being sensitive to vibrations and touch (Stettenheim, 1972; Seneviratne and Jones, 2008). Unlike mammalian hair, bird feathers have complex morphologies associated with function (Chuong *et al.*, 2000); including the well-developed plumulaceous and pennaceous contour feathers, and filoplumes, which have a long rachis and a tuft of barbs at the tip (Stettenheim, 2000). Avian rictal bristles have the simplest external structure of all feathers (Stettenheim, 1973). They are modified hair-like feathers composed of a single rachis, which sometimes also has venation at the base (Stettenheim, 1972). Feathers consist of 'dead cells' that are enclosed at their base by an innervated follicle, thus resembling mammalian whiskers (Lederer, 1972; Pass, 1989). While the function of mammalian whiskers is well described—they are prominent tactile sensors that are used to guide locomotion, navigation, foraging and social interactions (Mitchinson *et al.*, 2011; Grant and Arkley, 2016)—the function of rictal bristles remains unclear (Cunningham *et al.*, 2011).

Bristles may serve to increase the size of a bird's effective mouth gape (Van Tyne and Berger, 1976) or protect their eyes from airborne particles, prey items (Dyer, 1976; Conover and Miller, 1980) or vegetation (Brush, 1967; Hill, 1968). Rictal bristles may also prevent soiling of facial feathers during feeding (Chandler, 1914) or serve as a tactile organ to aid in navigation and obstacle avoidance (Küster, 1905; Lucas and Stettenheim, 1972). The position of the bristles above and along the upper beak, in front of the eye, may enable the bristles to 'bounce' flying insect legs or wings away from the eyes and into the mouth (Jackson, 2007). Experiments on Willow Flycatchers (*Empidonax traillii*) have revealed that rictal bristles can protect the eyes from prey particles, as taping around the bristles increased the number of small particles colliding with the eye (Conover and Miller, 1980). Experimental studies have also revealed that the rictal bristles of several other insectivorous neotropical tyrant-flycatchers (*Myiarchus crinitus, Sayornis phoebe, Contopus virens, Sarcophaga bullata, Empidonax traillii*) did not appear to play a role in prey capture, as these species captured prey items between the tip of the mandibles without the prey making contact with the bristles (Lederer, 1972; Conover and Miller, 1980). Conversely, it has been suggested that Brown Kiwis (*Apteryx mantelli*) use their bristles to guide nocturnal foraging for subterranean prey, due to their whiskers being sensitive to vibrations (Cunningham *et al.*, 2007, 2013). The prevalence of bristles in nocturnal and crepuscular bird species suggests that rictal bristles may play a role in navigation and obstacle avoidance in low-light conditions (Lucas and Stettenheim, 1972), but evidence for this is scant.

Anatomical studies conducted on the rictal bristles of owls (*Bubo bubo, Asio flammeus, Athene noctua* and *Strix aluco*) have revealed that their follicles are connected by muscle and connective tissue within the dermis, and are associated with nerves and mechanoreceptors (Küster, 1905; Stettenheim, 1973). Similarly, the follicles of the rictal bristles of Brown Kiwis are surrounded by Herbst corpuscles (Cunningham *et al.*, 2013), which are vibration-sensitive mechanoreceptors. The presence of Herbst corpuscles indicates that rictal bristles are likely to be sensitive to touch, airflow and vibrations. Feather follicles are connected to each other by several muscles, notably by the smooth apterial muscle (counteracting horizontal movements of feathers), the smooth erector muscle (which lifts the feather up) and the depressor muscle (which pulls the feather down and counteracts the vertical rotation of feathers induced by airflows) (Ostmann *et al.*, 1963). Bristle follicles may also be connected to each other and have the capacity to be mobile; however, bristle anatomy has been described in only a handful of bird species, and we know very little about rictal bristle mechanoreceptors and musculature. Further histological work is therefore required to describe rictal bristle anatomy and to test hypotheses about their function. In mammals, longer and more numerous whiskers are found in small, nocturnal, arboreal species. These species have regularly arranged whisker follicles with large, regular intrinsic muscles (Muchlinski *et al.*, 2013; 2020; Grant *et al.*, 2017); longer, thicker and stiffer whiskers also tend to have more numerous mechanoreceptors (Ebara *et al.*, 2002). Whereas the anatomy of mammalian whiskers is well described, the morphology and anatomy of avian rictal bristles are relatively unknown, and the relationship between bristle morphology and anatomy has not previously been investigated.

In this study, we focus on bird species belonging to the Order Caprimulgiformes, commonly referred to as nightjars, which are widely known for their nocturnal ecology, with species exhibiting a highly diverse range of foraging traits. Many Caprimulgiform species have obvious and prominent rictal bristles that vary in their shape, length, number and thickness. Here we describe the bristle morphology and follicle anatomy of 12 species, representing all five families and nine of the 22 genera of the traditional Caprimulgiformes (Cleere, 1998). We go on to describe follicle anatomy by identifying the presence and prevalence of mechanoreceptor Herbst corpuscles and provide muscle fibre descriptions. We explore associations with bristle morphology traits (bristle length, width and number) and discuss our findings in light of recent data on phylogenetic relationships and known foraging traits.

2 | MATERIALS AND METHODS

2.1 | Samples

Rictal bristle morphology and follicle anatomy were described in 12 species belonging to the Caprimulgiformes order in this study: fiery-necked nightjar (*Caprimulgus pectoralis*), pennant-winged nightjar (*Caprimulgus vexillarius*), European nightjar (*Caprimulgus europaeus*), spotted nightjar (*Eurostopodus argus*), Australian owlet-nightjar (*Aegotheles cristatus*), nacunda nighthawk (*Chordeiles nacunda*), common nighthawk (*Chordeiles minor*), tawny frogmouth (*Podargus strigoides*), large frogmouth (*Batrachostomus auritus*), Gould's frogmouth (*Batrachostomus stellatus*), oilbird (*Steatornis caripensis*), pauraque (*Nyctidromus albicolis*). *Caprimulgus pectoralis* and *Caprimulgus vexillarius* specimens were donated by Professor Tim Birkhead at the University of Sheffield; they were decapitated and preserved in formalin prior to their donation. All the other specimens were from the spirit collection at the Natural History Museum, Tring, UK, where they were dissected and preserved in 4% paraformaldehyde (PFA) in phosphate buffer solution (PBS). The rictal bristle region was fully intact in all specimens and thus representative of the morphological characteristics of each species, with the exception of *B. auritus*, whose bristles were damaged prior to collection. All work in this study was approved by the local ethics committee at Manchester Metropolitan University.

2.2 | Bristle morphology

For each species, the three longest rictal bristles were scanned on an Epson V600 scanner (12,800 dpi). Photos were taken using the Epson scan v 3.9.2 software, which gave calibrated measurements of the bristles. We were unable to photograph the bristles of *B. auritus* due to some of the bristles being damaged, and consequently we scanned only the base of rictal bristles for this species (see Figure 1). Bristle total length and bristle width at the base and tip were measured using the segmented line tool on ImageJ software from the scanned images. To compare the different morphotypes of bristles across species, we used three categories of bristle length: short (< 10 mm), medium (15–30 mm) and long (> 30 mm). Bristle width was also classified into three categories: thin (< .3 mm), moderately thick (.3–.6 mm) and thick (> .6 mm). Bristle number was counted from the rictal region of two individual specimens per species from the skin collection within the Natural History Museum, Tring, and Liverpool World Museum. As the number of bristles did not vary between each side of the head of each specimen, we only conducted bristle counts on one side of the face. Bristle numbers were categorised into few (≤ 10) and many (> 10).

2.3 | Follicle anatomy

2.3.1 | Dissection and histology

We define the rictal region as the patch of skin from the rictus to nares, at the base of the upper mandible of the beak (Figure 2). Subsequently, we defined all bristles collected from this area as rictal bristles. All other (non-bristle) feathers were trimmed from the rictal region of each specimen. The rictal bristle region was removed from one side of the head by cutting a 5-mm-wide piece of skin along the length of the upper beak and across the head near the nare using a scalpel. There was noticeable variation in bristle positioning across all specimens examined and consequently the shape and position of the dissected sections varied (Figures 4B, 5D, 6C and 7C,D). Rictal bristles were removed from the skin of each sample by cutting them flat to the skin sample; the base of the bristle was kept within the follicle to allow the follicle to maintain its form during slicing and staining.

Skin tissue was flattened for 5 hr between two sponges in histology cassettes in 70% industrial methylated spirit (IMS). Tissue samples were loaded into a tissue processor (Shandon Citadel 2000) to dehydrate through a graded series of ethanol (70, 80, 90 and 100%) and xylene baths and infiltrated with paraffin wax, in a process lasting approximately 20 hr. Tissue was then embedded in solid blocks of paraffin that were sliced at 10 µm on a rotary microtome (Thermo Scientific Microm HM355S) with water bath (37°C), and mounted onto slides, which were stained with standard Masson's Trichrome staining and cover-slipped with DPX mountant. Microscope images were taken using Zeiss Zen Pro imaging software on a Zeiss AxioImager M1 Brightfield microscope and AxioCam HMRc.

2.3.2 | Characterisation of follicle anatomy

For each species, components of the follicle and immediate surroundings of the follicle were identified and characterised from the microscope images, including describing the tissue type, musculature and mechanoreceptors. For each species, the dermis density and the adipose tissue quantity were quantified as possible predictors of tissue quality and functionality, using the following coding system. The quantity of adipose tissue was expressed as the percentage of space allocated for adipose tissue within the integument section imaged. Dense tissue was here defined as having ≤ 40% adipose tissue, and *porous* tissue was defined as having > 40% of adipose tissue within the histology section. Dense tissue may correspond to a higher quality of tissue, containing more sensory components. For each species, the presence and type of intrinsic fibres and mechanoreceptors were identified from the dermis. Types of intrinsic fibres were determined following the feather muscle description by Homberger and De Silva (2000) and defined as smooth apterial muscle, and erector or depressor feather muscle (the histology sections showed the characteristic diagonal fibres of the erector and depressor muscles, running from the tip of a feather follicle to the base of the neighbouring one, but the angle of the sections did not permit us to identify which of the depressor or erector muscles was present on the picture). The size of the fibre bundles was categorised by counting the number of fibres attached together in the area immediately surrounding the follicle; with ≥5 adjacent fibres considered a 'large fibre bundle', < 5 considered a 'small fibre bundle', and NA if no fibres were present. The presence/absence of mechanoreceptors was documented and, where present, the number of mechanoreceptors was counted per follicle and position. Based on our findings, we defined the number of Herbst corpuscles as low when their number around a follicle was < 7 and high when ≥ 7. Mechanoreceptor position was described as 'base' when they were only apparent at the proximal tip (base) of the bristle follicle, or 'around' when they were recorded from the base and the sides of the follicle. Mechanoreceptors were identified as Herbst corpuscle following Gottschaldt (1985). It was not possible to measure the depth of the bristle follicle due to variation in the orientation of the follicles within the tissue sample and the resulting histology slices.

2.3.3 | Relationship between bristle morphology, anatomy and foraging traits

Phylogenetic relationships of all nightjar species based on rictal bristle morphotypes (cf. drawings on phylogenetic branches in Figure 2) were represented using a phylogenetic tree from Birdtree.org (http://birdtree.org, tree set: HackettStage2Full, sample size: 1,000). Two k-means cluster analyses were conducted in MATLAB (MATLAB and Statistics Toolbox Release 2019a, The MathWorks, Inc.) to identify morphotypes from our bristle morphology and follicle anatomy descriptors. The bristle morphology variables included the discrete categories of bristle length, width, number and branching, and the follicle anatomy variables included the discrete categories of muscle bundle size, tissue density and Herbst corpuscle number. Both datasets were partitioned into five defined groups using k-means distance measures. Relationships between the numbers of Herbst corpuscles, bristle length, width and number were visualised using the package *ggplot2* (Wickham, 2009) in R software, version 3.5.0 (R Core Team, 2016) and analysed using non-parametric Spearman's rank correlations (due to low sample sizes and skewed data). All values are presented as mean values ± SD with significance level assumed at $p < .05$. Five ecological traits from Cleere (1998) were identified for each sampled species (Table S1) to compare and discuss these ecological traits in association with bristle morphology and follicle anatomy: foraging time, diet, foraging method, foraging height and habitat density. The traits are defined in detail in Table S1.

3 | RESULTS

3.1 | Bristle morphology

All three frogmouth species (*P. strigoides*, *B. auritus* and *B. stellatus*) had rictal bristles of medium length with a thick bristle base and a high number of bristles (Figure 2), which were conspicuously branched in structure (Figure 1). *Podargus strigoides* and *B. auritus* bristles were branched throughout with barbs and barbules, especially at the base, whereas *B. stellatus* had simple branching bristles with numerous barbs throughout (Figure 1). *Caprimulgus pectoralis*, *C. vexillarius*, *C. europaeus* and *N. albicollis* had medium length bristles with unbranched rictal bristles that occurred in lower numbers (≤10 bristles per side of the face) than the other species in the study (Figures 1 and 2). In contrast, *C. nacunda*, *C. minor* and *E. argus* had the thinnest and shortest rictal bristles, occurring in high numbers, and were branched with barbs (Figures 1 and 2). In all species with branched rictal bristles, *C. nacunda* had the least number of barbs on the shaft, from base to tip (Figure 1). *Aegotheles cristatus* had similar bristle lengths and widths to *C. pectoralis*, *C. vexillarius*, *C. europaeus* and *N. albicollis* (all with medium length and width); however, its bristles were a combination of branched with barbs from the base to the tip of the bristles and unbranched bristles, and had relatively lower bristle numbers (Figures 1 and 2). Finally, *S. caripensis* had thick, long, unbranched rictal bristles that curved downwards, in contrast to the bristles on all the other species, which curved slightly upwards at the distal tip (Figures 1 and 2), and had a high number of rictal bristles (15 bristles ± 1.4 SD) (Figure 2). Thus in our 12 species, bristle morphology could be visually summarised by five different morphotypes (coloured purple, green, blue, brown and orange in Figure 2), as confirmed by a k-means cluster analysis (Table S2). Combining data from all the species sampled, bristle length and width were not correlated

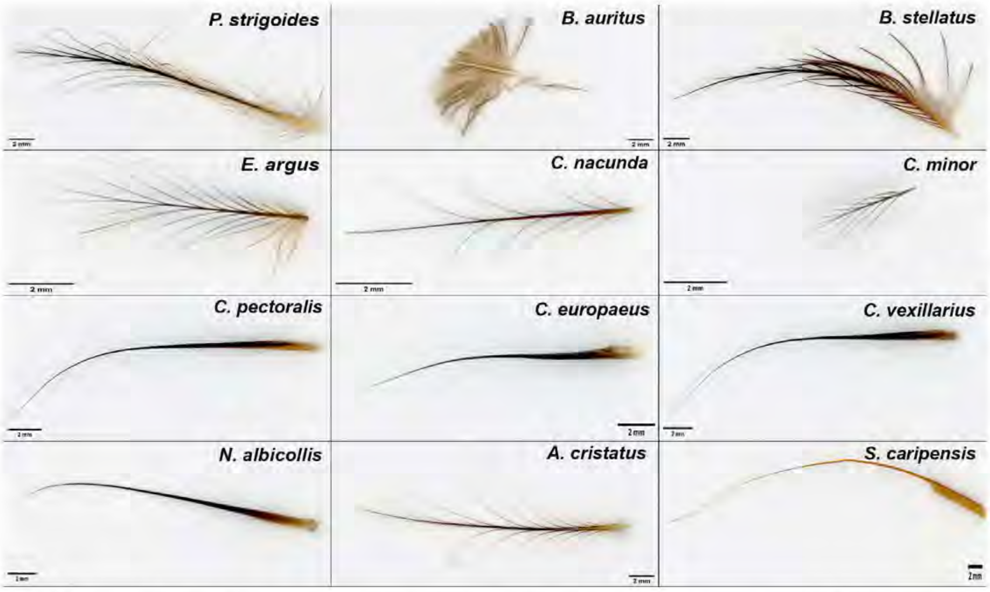


FIGURE 1 Scan of 12 rictal bristles showing the different bristle morphologies present in the Caprimulgiform order. Rictal bristles illustrated are from the longest rictal bristles found for each of our 12 species. Scale bar: 2 mm

(Spearman's rank correlation: *rho* = .36, *p* = .27; Figure S1), nor was bristle length correlated with bristle number (Spearman's rank correlation: *rho* = .05, *p* = .88). However, bristle number and bristle width were correlated (Spearman's rank correlation: *rho*= −.67, *p* = .016; Figure S1), suggesting that species with more bristles present, such as *C. nacunda*, *C. minor* and *E. argus*, also had much thinner bristles.

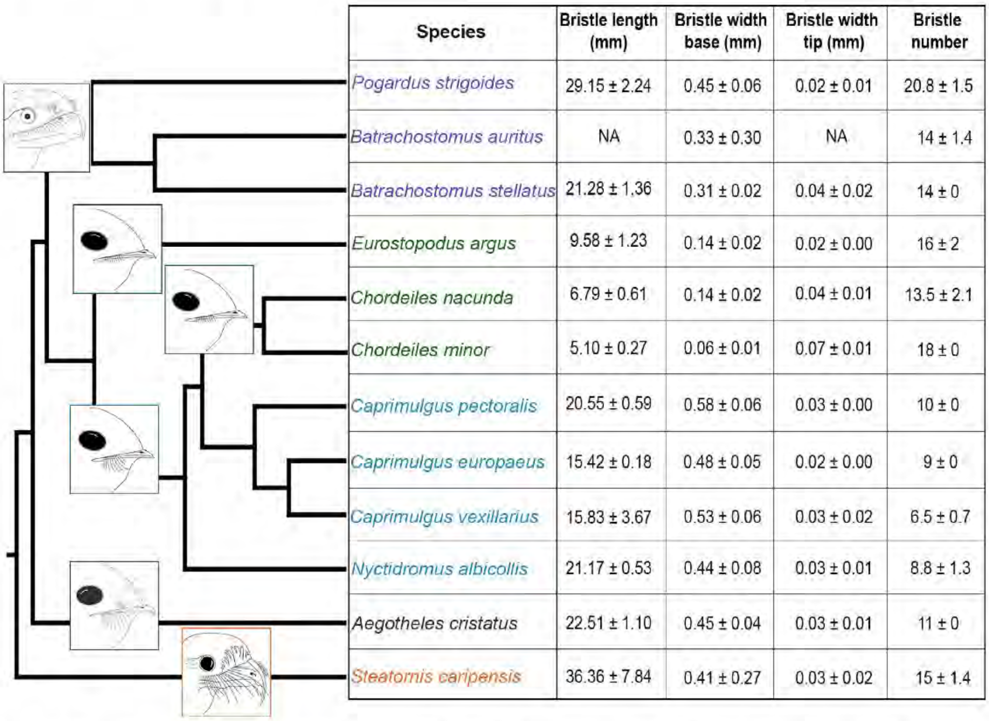
3.2 | Follicle anatomy

3.2.1 | Characterisation of follicle anatomy

Staining of the skin of the rictal region revealed high inter-species variability of the bristle-bearing integument, especially in the presence of Herbst corpuscle mechanoreceptors (Figure 3) and the intrinsic fibres in the surrounding of the bristle follicles (Table 1). There was no clear pattern in follicle anatomy morphotypes, based on a k-means cluster analysis (Table S2).

The bristle-bearing integument of *P. strigoides*, *B. auritus* and *B. stellatus* revealed a dense tissue (Figure 4, Table 1) with the smooth erector or depressor muscles running diagonally from consecutive bristle follicles. In *P. strigoides* and *B. auritus*, the smooth apterial bristle muscle was also present, joining the distal tips of bristles together (Figure 4). In all three specimens, the dermis contained connective tissue around the rictal bristle follicles (shown as a blue ring around the follicle; Figure 4). Fewer Herbst corpuscles mechanoreceptors were present in the surroundings of the rictal bristle follicles (Figure 4, Table 1). Herbst corpuscle structure possessed a characteristic outer capsule enclosing an onion-shaped lamellate outer zone and an inner bulb, surrounded by two rows of Schwann cell nuclei (Figure 3).

The integument of *C. nacunda*, *C. minor* and *E. argus* revealed porous tissue within the dermis (Figure 5, Table 1). Samples from all three species had bristle follicles enclosed in connective tissue (Figure 5). *Chordeiles nacunda* had no intrinsic fibres present in the surroundings of the bristle follicles, but *C. minor* and *E. argus* had



Species	Bristle length (mm)	Bristle width base (mm)	Bristle width tip (mm)	Bristle number
Pogardus strigoides	29.15 ± 2.24	0.45 ± 0.06	0.02 ± 0.01	20.8 ± 1.5
Batrachostomus auritus	NA	0.33 ± 0.30	NA	14 ± 1.4
Batrachostomus stellatus	21.28 ± 1.36	0.31 ± 0.02	0.04 ± 0.02	14 ± 0
Eurostopodus argus	9.58 ± 1.23	0.14 ± 0.02	0.02 ± 0.00	16 ± 2
Chordeiles nacunda	6.79 ± 0.61	0.14 ± 0.02	0.04 ± 0.01	13.5 ± 2.1
Chordeiles minor	5.10 ± 0.27	0.06 ± 0.01	0.07 ± 0.01	18 ± 0
Caprimulgus pectoralis	20.55 ± 0.59	0.58 ± 0.06	0.03 ± 0.00	10 ± 0
Caprimulgus europaeus	15.42 ± 0.18	0.48 ± 0.05	0.02 ± 0.00	9 ± 0
Caprimulgus vexillarius	15.83 ± 3.67	0.53 ± 0.06	0.03 ± 0.02	6.5 ± 0.7
Nyctidromus albicollis	21.17 ± 0.53	0.44 ± 0.08	0.03 ± 0.01	8.8 ± 1.3
Aegotheles cristatus	22.51 ± 1.10	0.45 ± 0.04	0.03 ± 0.01	11 ± 0
Steatornis caripensis	36.36 ± 7.84	0.41 ± 0.27	0.03 ± 0.02	15 ± 1.4

FIGURE 2 Phylogenetic tree for the species belonging to the Caprimulgiformes order used in this study, combined with a table showing their bristle measurements. Drawings showing the bristle morphology and position on the beak were added on the tree for better visualisation, and also to illustrate the head and beak shapes of the species. The tree was created by BirdTree.org. Measurements were taken from scanned bristles from each species and included the mean bristle length, mean bristle width at the base and at the tip (± standard deviation). Species names are colour-coded following their bristle morphotypes: frogmouths in purple, nighthawks and spotted nightjar in green, nightjars and pauraque in blue, Australian owlet-nightjar in brown, and oilbird in orange

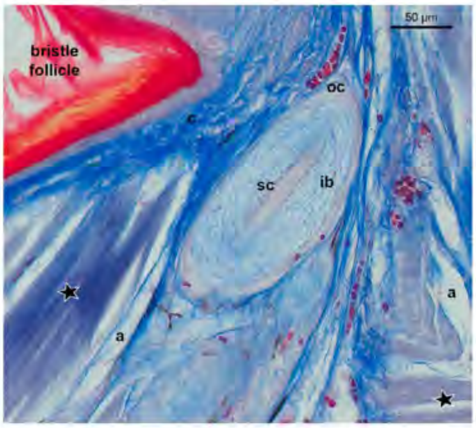


FIGURE 3 Longitudinal section of the dermis layer showing a cross-section of Herbst corpuscles around a bristle follicle from the skin region above the upper beak of *C. pectoralis*. Star: muscle fibres. (a) adipose tissue; (c) connective tissue; (oc) outer capsule; (ib) inner bulb; (sc) rows of Schwann cells. Sections stained with Masson's trichrome stain

small bundles of the smooth erector or depressor muscle fibres extending from the base of a follicle to the neck of a neighbouring one (Figure 5, Table 1). There were no mechanoreceptors present in the tissue immediately surrounding the rictal bristles in *C. nacunda* or *C. minor*, whereas a high number of Herbst corpuscles were found in the tissue surrounding the rictal bristle follicles in *E. argus* (Figure 5, Table 1).

There was interspecific variation in the musculature, mechanoreceptors and density of the bristle-bearing integument in *C. pectoralis*, *C. vexillarius*, *C. europaeus* and *N. albicollis* (Figure 6). *Caprimulgus pectoralis* displayed a dense tissue (Figure 6A, Table 1), with a dermis presenting rictal bristle follicles connected together by a dense layer of smooth apterial muscle (Figure 6A). A high number of mechanoreceptors were present all around the rictal bristle follicles in *C. pectoralis* from the distal tip of the follicle (Figure 6A, Table 1). The skin tissue of *C. vexillarius* revealed a porous integument, in which small bundles of smooth erector or depressor muscles fibres connected the bristle follicles together (Figure 6B; Table 1). There was no evidence of mechanoreceptors in the bristle-bearing integument of *C. vexillarius* (Figure 6B, Table 1). The integument of *C. europaeus* consisted of a porous dermis with a small bundle of smooth erector or depressor muscle fibres that appeared

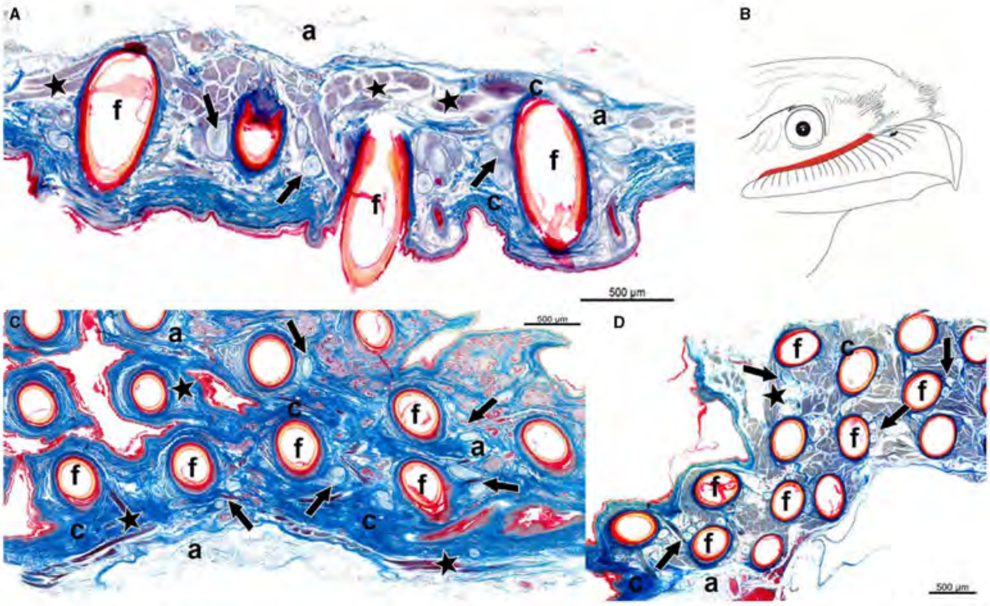


FIGURE 4 Longitudinal section of the dermis layer showing a cross-section of bristle follicles from the skin region above the upper beak of (A) *P. strigoides*, (C) *B. auritus* and (D) *B. stellatus*, from the nares to the rictus (right to left). Sections revealed the presence of mechanoreceptors, i.e. Herbst corpuscles (arrows), muscle fibres (stars), and adipose tissue (a) surrounding the bristle follicles (f). Sections stained with Masson's trichrome stain. (B) Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *P. strigoides*, *B. auritus* and *B. stellatus*

to connect bristle follicles together, and a thin layer of connective tissue was apparent at the base of the bristle follicles (Figure 6D; Table 1). In addition, a small number of Herbst corpuscles were present around the rictal bristle follicles, constricted at the base of the follicle (Figure 6D, Table 1). The integument of *N. albicollis* revealed dense tissue and a large bundle of smooth erector or depressor muscle fibres, pairing bristle follicles together (Figure 6E, Table 1). The follicles were also surrounded by connective tissue, in which the muscle fibres seemed to be attached. Herbst corpuscles were present all around the follicles, from the distal tip to the neck of the follicle (Figure 6E); however, the number of mechanoreceptors in *N. albicollis* was low compared with the other species (Figure 6E, Table 1).

The sections from the *A. cristatus* specimen had a different orientation (angle) to the other samples and did not show the same longitudinal section of the bristle follicle. *Aegotheles cristatus* had dense tissue (Figure 7A, Table 1), with large bundles of the smooth erector or depressor muscle fibres, pairing the bristle follicles together. Furthermore, the bristle follicle appeared to be surrounded by connective tissue and Herbst corpuscles (Figure 7A). Despite the different orientation (angle), a high number of Herbst corpuscles around the follicles were distinguishable (Figure 7A, Table 1). The integument of *S. caripensis* consisted of a dense tissue, with a large sheet of striated subcutaneous muscle fibres underlying the bristle follicles (Figure 7B, Table 1). *Steatornis caripensis* had abundant connective tissue around the follicle, as well as numerous Herbst corpuscles (Figure 7B, Table 1).

3.2.2 | Relationship between bristle morphology and follicle anatomy

Herbst corpuscle number was not significantly correlated with bristle length (Spearman's Rank: *rho* = .39, *p* = .23) or bristle width (Spearman's rank correlation: *rho* = .23, *p* = .47) (Figure S1). However, *S. caripensis*, which had the longest bristles, also had the highest number of mechanoreceptors, while in the two *Chordeiles* species, which had the shortest and thinnest bristles, had no mechanoreceptors present (Table 1). While we were not able to test this statistically, small bundles of muscle fibres tended to be present in species with short (*C. minor* and *E. argus*) and medium-sized bristles (*C. vexillarius* and *C. europaeus*), whereas large muscle bundles were present in species with medium or long bristles (Table 1). Herbst corpuscle number was not correlated to bristle number (Spearman's rank correlation: *rho*= −.03, *p* = .93; Figure S1). Therefore, although there are some anecdotal associations of bristle morphology and follicle anatomy in our data, quantitative measurements of bristle length, width and number were not correlated with the number of Herbst corpuscles.

4 | DISCUSSION

4.1 | Bristle morphology

We identified five different bristle morphotypes in the 12 Caprimulgiformes species investigated in this study (Figure 2,

TABLE 1 Summary table of the anatomical descriptions of the bristle-bearing integument of the 12 species of the Caprimulgiform order

Species	Adjacent muscle	Fibre bundle	Connective tissue	HC position	HC number	Dermis density	Quantity of adipose tissue, %	Bristle number
P. strigoides	Smooth erector/depressor muscle smooth apterial muscle	Large	Yes	Around	4	Dense	20	20.8 ± 1.5
B. auritus	Smooth erector/depressor muscle smooth apterial muscle	Large	Yes	Around	5	Dense	10	14 ± 1.4
B. stellatus	Smooth erector/depressor muscle	Large	Yes	Around	5	Dense	30	14 ± 0
C. nacunda	Absent	Absent	Yes	Absent	0	Porous	70	13.5 ± 2.1
C. minor	Smooth erector/depressor muscle	Small	Yes	Absent	0	Porous	70	18 ± 0
E. argus	Smooth erector/depressor muscle	Small	Yes	Around	10	Porous	60	16 ± 2
C. pectoralis	Smooth apterial muscle	Large	Yes	Around	10	Dense	40	10 ± 0
C. vexillarius	Smooth erector/depressor muscle	Small	Yes	Absent	0	Porous	80	6.5 ± 0.7
C. europaeus	Smooth erector/depressor muscle	Small	Yes	Base	6	Porous	60	9 ± 0
N. albicollis	Smooth erector/depressor muscle	Large	Yes	Around	5	Dense	20	8.8 ± 1.3
A. cristatus	Smooth erector/depressor muscle	Large	Yes	Around	8	Dense	40	11 ± 0
S. caripensis	Striated subcutaneous muscle	Large	Yes	Around	10	Dense	10	15 ± 1.4

Note: HC position corresponds to the position of Herbst corpuscles at the bristle follicle. Bristle number corresponds to the number of bristles on one side of the face. Species names colour-coded following their bristle morphotypes, based on bristle size (length and width), shape (branching present and absent) and number (not follicle anatomy): frogmouths in purple, nighthawks and spotted nightjar in green, nightjars and pauraque in blue, Australian owlet-nightjar in brown, and oilbird in orange.

HC, Herbst corpuscles.

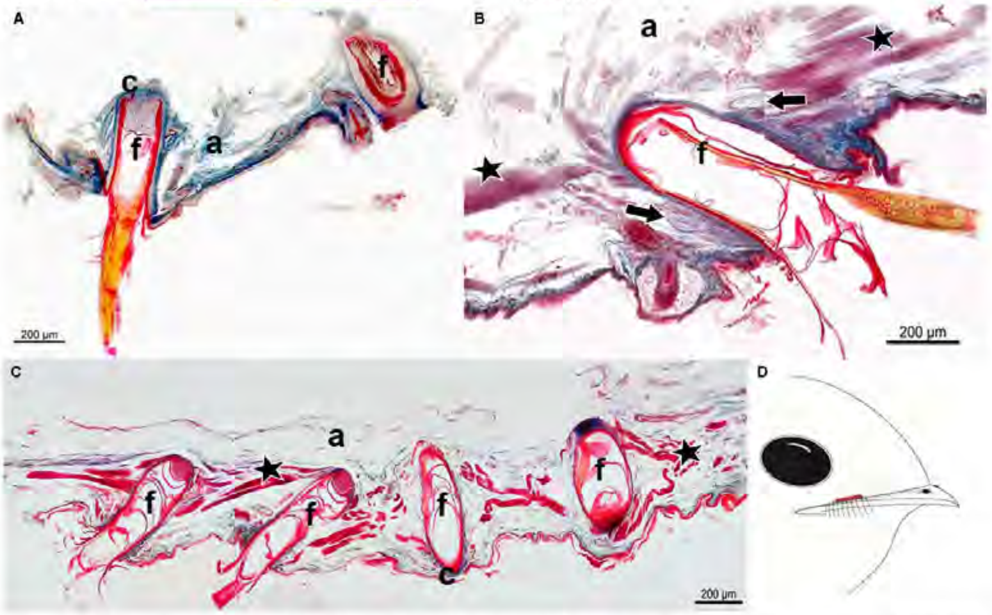


FIGURE 5 Longitudinal section of the dermis layer showing a cross section of bristle follicles from the skin region above the upper beak of (A) *C. nacunda*, (B) *E. argus* and (C) *C. minor*, from the nares to the rictus (right to left). Sections revealed the presence muscle fibres (stars), adipose tissue (a), and mechanoreceptors, i.e. Herbst corpuscles (arrows) only in *E. argus*, surrounding the bristle follicles (f). Sections stained with Masson's trichrome stain. (D) Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *C. nacunda*, *C. minor* and *E. argus*

Table S2), which varied in bristle size (length and width), shape (branching present and absent) and number. These morphotypes seemed to be in accordance with a contemporary Caprimulgid phylogeny (Figure 2, Table S2), where closely related species had similar bristle morphologies. We also noticed that the beak and head shapes of the species affected the position of the bristles, and hence the dissection area (Figures 4-7). Beak shape is one of the most diverse traits in birds, varying in morphology according to individual species and their feeding strategies (Stettenheim, 2000; Cunningham *et al.*, 2013; Thomas *et al.*, 2016). Due to the small sample sizes and specimen availability of the species in this study, we are not able to conduct phylogenetic analyses; however, we demonstrate below that bristle morphology does not coincide consistently with beak shape and foraging traits, rather it is distributed non-randomly over the contemporary Caprimulgid phylogeny.

Seven of our sampled species (*C. pectoralis*, *C. vexillarius*, *C. europaeus*, *N. albicollis*, *C. nacunda*, *C. minor* and *E. argus*) are predominantly insectivorous, with small, thin beaks and a wide mouth gape (Cleere, 1998). However, although the aforementioned species share a similar beak shape and diet, they vary widely in their bristle morphology, including length, width and branching. Hence, *C. pectoralis*, *C. vexillarius*, *C. europaeus* and *N. albicollis* appear to be in a group distinct from *C. nacunda*, *C. minor* and *E. argus*, in terms of both bristle morphology and phylogenetically. Although *A. cristatus* has the same diet as the seven previous species, it has a slightly more keratinised and hooked beak than the others (Cleere, 1998) and possesses a combination of unbranched and branched bristles. There are also examples in our specimens where a congruence seems to appear between bristle morphology, beak shape, foraging traits and phylogeny. *Podargus strigoides*, *B. auritus* and *B. stellatus* all shared similar beak shape, diet (Cleere, 1998) and bristle morphology and thus form a sister group, as these features differed from our other species (Figure 2). *Steatornis caripensis* has a different beak shape, diet (Cleere, 1998) and bristle morphology than all the other species (Figure 2), consequently *S. caripensis* is rather distinct from the others and is on its own within the phylogeny. Measuring the bristle morphology of more specimens and species will allow us to explore these relationships further, especially using phylogenetic analyses.

4.2 | Follicle anatomy

For all species examined in this study, we found that bristle follicles were anchored in the dermis of the skin with an outermost layer of connective tissue, which is consistent with previous studies on

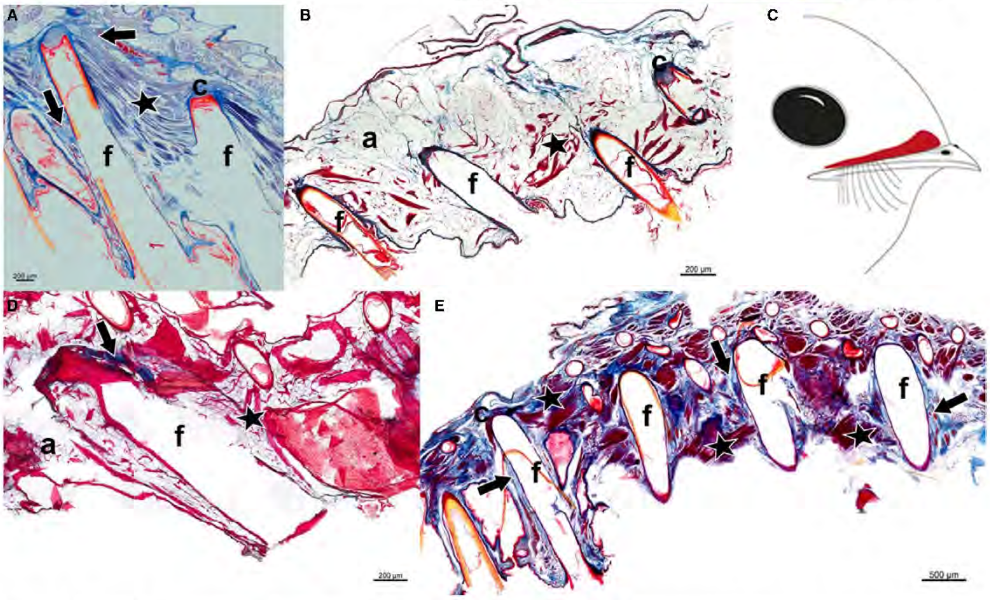


FIGURE 6 Longitudinal section of the dermis layer showing a cross section of bristle follicles from the skin region above the upper beak of (A) *Caprimulgus pectoralis*, (B) *Caprimulgus vexillarius*, (D) *Caprimulgus europaeus* and (E) *Nyctidromus albicollis*, from the nares to the rictus (right to left). Sections revealed the presence of mechanoreceptors, i.e. Herbst corpuscles (arrows), muscle fibres (stars), and adipose tissue (a) surrounding the bristle follicles (f). Sections stained with Masson's trichrome stain. (C) Schematic drawing illustrating in red the correspondent skin region cut along the upper beak, enclosing the bristle follicles of *P. strigoides*, *B. auritus* and *B. stellatus*

avian rictal bristles (Ostmann *et al.*, 1963; Homberger and De Silva, 2000). The appearance of muscle fibres connecting the follicles has also been observed in contour feathers, such as in the wild turkey (*Meleagris gallopavo*) (Homberger and De Silva, 2000; 2003). The size and arrangement of smooth muscles at the follicles have been found to vary between feather types, i.e. large flight feathers have larger muscle bundles then small filoplumes, which have no muscles (Stettenheim, 2000). In agreement with this latter study, we also found that short, thin bristles had smaller muscle bundles than medium or long, wide bristles (Figure 2, Table 1).

Bristle morphology was not associated with follicle anatomy in the species in this study (Figures 1-7), as there was no correlation between the presence of muscles and the number of Herbst corpuscles, and bristle length, width or number. In mammalian whiskers, whisker length, width and number are associated with aspects of follicle anatomy and musculature (Muchlinski, 2010; Muchlinski *et al.*, 2013; Grant *et al.*, 2017); specifically, longer and more numerous whiskers tend to be associated with more arranged follicles and large intrinsic muscles (Muchlinski, 2010; Muchlinski *et al.*, 2013; Grant *et al.*, 2017), and longer, thicker and stiffer whiskers tend to have numerous mechanoreceptors (Ebara *et al.*, 2002). However, this was not the case in our species. For example, *C. pectoralis*, *C. vexillarius* and *C. europaeus* had differences in follicle anatomy from one another, in terms of the size of muscle fibre bundles and mechanoreceptors, although they had the same bristle morphology. While bristle morphology visually agreed with the contemporary Caprimulgiform phylogeny (Figures 1 and 2), the follicle anatomy (musculature and Herbst corpuscle number) did not. If this is the case, bristle follicle anatomy, and hence tactile sensitivity, may be better explained by life-history traits, rather than phylogenetic relatedness. This has not been explored across a comparative dataset before and should form the basis of future studies on bristle anatomy and morphology.

4.3 | Foraging traits and bristle function

The Caprimulgiform order is ecologically and behaviourally diverse, with species exhibiting different activity patterns, diet, foraging methods, foraging niches and patterns of habitat selection (Table S1) (Cleere, 1998). We suggest below that foraging time, foraging habitat selection and foraging method might all be associated with follicle anatomy in the Caprimulgiformes species that we studied here; specifically, tactile sensitive rictal bristles (with mechanoreceptors) are present in the Caprimulgiform species that forage in scotopic conditions in more closed, structurally complex habitats, whereas bristles without mechanoreceptors are present in our Caprimulgiformes species that are open habitat, partially diurnal foragers.

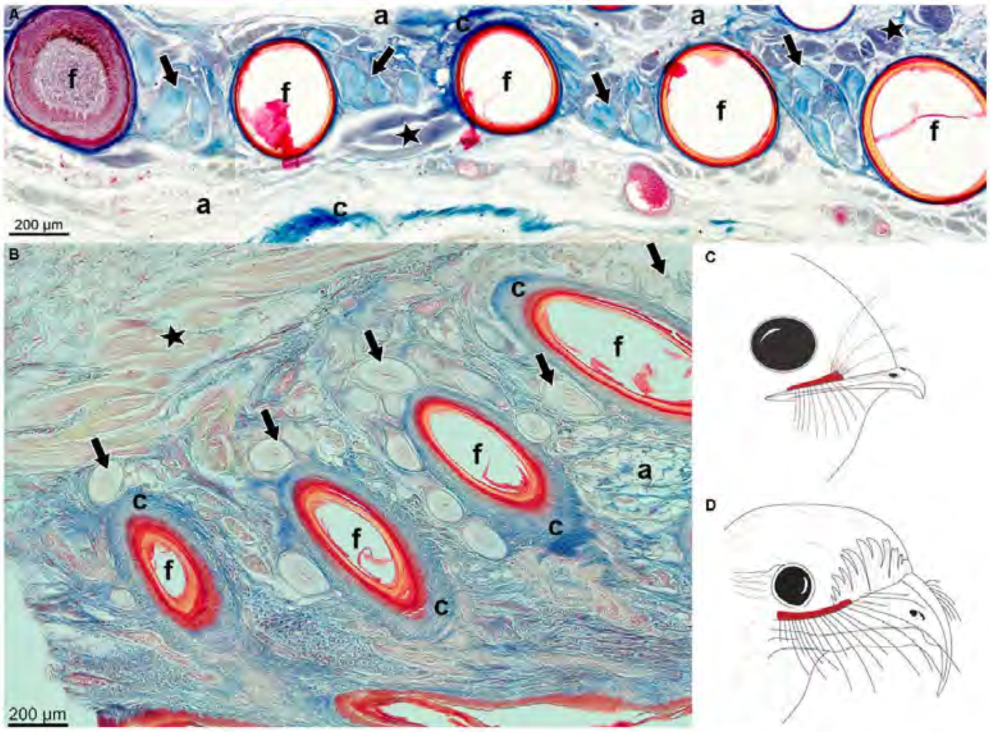


FIGURE 7 Cross- (A) and longitudinal section (B) of bristle and feather follicles from the dermis layer of the rictal region above the upper beak of (A) *Aegotheles cristatus* and (B) *Steatornis caripensis*, from nare to rictus (right to left). Sections revealed the presence of mechanoreceptors, i.e. Herbst corpuscles (arrows), muscle fibres (stars), and adipose tissue (a) surrounding the bristle follicles (f). Sections stained with Masson's trichrome stain. Schematic drawing illustrating in red the skin region cut along the upper beak, enclosing the bristle follicles of (C) *A. cristatus* and (D) *S. caripensis*

Three of our study species that forage pre-dusk (*C. nacunda*, *C. minor* and *C. vexillarius*) had no mechanoreceptors present around their rictal bristle follicles, and had only short to medium length bristles (Figures 5A,C and 6B Table 1). The anatomy morphotype k-means cluster analysis also grouped these species together (Table S2). Although *C. vexillarius* is found in semi-open habitats, whereas *C. nacunda* and *C. minor* are found in open habitats, all three species forage by hawking high above the canopy (in the case of *C. vexillarius*) or in open country or riverbeds (in the case of *C. nacunda* and *C. minor*) (Tables 1 and S1; Holyoak, 2001). Therefore, open-habitat, partially diurnal foraging species seem to have reduced bristle tactile function. The lack of mechanoreceptors suggests that these species may rely more on vision than touch, as flying during daylight in open habitats probably makes them less likely to collide with obstacles and more likely to detect prey visually. Rictal bristles in these species may still play a role in eye protection against flying items during feeding, despite them being less than 20 mm in length (Lederer, 1972; Conover and Miller, 1980). In addition, the bristles may also still be sensitive, as their movement could be detected in the absence of Herbst corpuscles by other mechanoreceptors, e.g. stretch receptors such as Ruffini corpuscles, which detect pressure and tension and are present in the muscle and skin (Halata and Munger, 1980).

Contrary to these partially diurnal species, we found that all nine obligate crepuscular and nocturnal species had mechanoreceptors present around their bristle follicles and so are likely to have a tactile function. These species all forage in complex or closed habitats, which suggests that bristles may be involved in foraging, navigation and collision avoidance in the dark. The evolution of avian foraging traits often depends upon a trade-off between vision and other sensory systems (Cunningham *et al.*, 2013; Corfield *et al.*, 2015), therefore, touch sensing might well be more developed in nocturnally foraging species, as is the case in mammals (Muchlinski, 2010; Muchlinski *et al.*, 2013). The number of mechanoreceptors located around the follicle varied across our species, and we observed no clear relationship between the number of Herbst corpuscles and the anatomy morphotypes, with their foraging traits—the status of obligate crepuscular and nocturnal, and the density of their habitat (Table S1). This indicates that the sensitivity of rictal bristles is likely to be dependent upon a combination of species-specific life-history traits. For example, two obligate nocturnal species, *B. auritus* and *B. stellatus*, which prefer close habitats such as densely vegetated forest habitats, had low numbers of mechanoreceptors, whereas near-obligate nocturnal species *C. pectoralis*, *S. caripensis* and *E. argus*, had the highest number of Herbst corpuscles present, and live in both semi-open and closed habitat. The near-obligate nocturnal *C. europaeus*, which prefers semi-open habitat (i.e. a mixture of open country and woodlands, Table S1), had low numbers of mechanoreceptors present at the base of the bristle follicle (Table S1). *Caprimulgus europaeus*, like the partially diurnal species that lacked mechanoreceptors, is also an aerial hawking species (Cleere, 1998). Therefore, sensitive, tactile rictal bristles may not be beneficial for species that predominantly feed by aerial hawking.

Feathers have previously been found to guide navigation and foraging in a number of species. For example, the crest and super-orbital plumes in whiskered auklets (*Aethia pygmaea*) are used to detect obstacles and guide navigation in dark crevices (Seneviratne and Jones, 2008). Brown kiwi (*Apteryx mantelli*) are nocturnal birds that have long rictal bristles, with a mean of 36.63 mm (SD 7.97, $n = 1$ individual, 6 bristles) (M. G. Delaunay unpubl. data), which are longer than any of the bristles in our study species, and also possess numerous (>10) Herbst corpuscle mechanoreceptors positioned all around the follicles (Figure 3 in Cunningham *et al.*, 2011), which is similar to the number in *E. argus*, *C. pectoralis* and *S. caripensis* in our study (Figures 5B, 6A and 7B). Cunningham *et al.*, (2011) suggest that, as kiwis probe-forage holding their bristle forwards, they may use their tactually sensitive rictal bristles to detect subterranean prey or to assess the quality of the foraging ground. Therefore, rictal bristles of *E. argus*, *C. pectoralis* and *S. caripensis* may be functionally similar and help to detect flying prey or obstacles during flight. Corfield *et al.* (2014) also proposed that brown kiwi rictal bristles are tactile and might help with navigation in the dark.

Defining the function of rictal bristles is complex and their sensitivity is likely to be dependent upon a combination of species-specific life-history traits. This is the case in mammalian whiskers, where aquatic mammals have more sensitive whiskers with 10× more nerve endings than terrestrial mammals (Hyvärinen and Katajisto, 1984; Hyvärinen, 1989; Marshall *et al.*, 2006), whereas arboreal, nocturnal small mammals have larger and more regular whisker muscles (Grant and Arkley, 2016; Muchlinski *et al.*, 2020); although many aspects of muscle and whisker arrangements can still vary on a species-by-species basis (Yohro, 1977; Grant *et al.*, 2013, 2017). Further work investigating more species and specimens across Caprimulgiformes and other avian orders will help to develop our understanding of bristle function, specifically addressing which ecological variables might predict the presence of long, sensitive bristles. However, the morphology and anatomy of the rictal bristles are not closely associated. This means that morphological variables cannot infer tactile sensitivity, and conducting large-scale morphological studies, using museum skin collections for instance, will have more limited applications. Rictal bristles are present in many avian species, not only in the Caprimulgiformes, and investigating the bristle morphology and follicle anatomy in more diverse species could help us better define the function and evolution of rictal bristles.

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AUTHOR'S CONTRIBUTIONS

R.G. and M.D. designed the study and drafted the manuscript. M.D. performed the histology, did the measurements and made the figures. C.L. helped getting access to the spirit collection of the Natural museum of Tring and the skin collection of the World museum of Liverpool. Analyses and interpretations were performed by M.D. and critically reviewed by R.G., C.L., H.L. and M.S. All authors revised the manuscript and provided final approval before submission.

DATA AVAILABILITY STATEMENT

We have supplied the data for each species (mean and SD); these are available in the tables of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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