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Research Article:

Light and electron morphological studies on the upper beak of the little owl (*Athene noctua*)

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Abstract

The present study investigated the morphological features of the upper beak of the little owl *(Athene noctua)* using gross anatomy, scanning electron microscopy, and light microscopy. The upper beak is tough, with a wide base and a strongly curved, sharp-pointed tip. The upper beak gradually increases in both width and height. Scanning electron microscopy revealed that both the tip and lateral edges of the upper beak are covered with numerous irregular keratinized flakes. The upper beak of the little owl consists of a bony basis covered by dermal and epidermal layers of varying thickness. The bony basis is composed of premaxillary bones in the rostral and lateral regions. The premaxillary bone is covered by a keratinized stratified squamous epithelium comprising multiple layers: the stratum basale, stratum spinosum, stratum transitivum, and stratum corneum. The dermis is composed of dense irregular connective tissue containing blood vessels and nerves. The dermis is rich in small capillaries and sensory corpuscles, primarily Herbst corpuscles.

1. Introduction

The little owl (*Athene noctua*) is a carnivorous bird classified within the Order Strigiformes and Family Strigidae (Scopoli, 1769). Its diet comprises various prey items, including insects such as beetles grasshoppers, and other arthropods, small reptiles, amphibians, worms, mammals, and birds. This species exhibits a wide geographic distribution, extending from Denmark southward to North Africa and eastward to Asia. Additionally, it was introduced to New Zealand and England, where it subsequently spread to Scotland and Wales (Mikkola, 2013).

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As birds lack both lips and teeth, the beak serves as a multifunctional tool (Gelis, 2006; Gofur, 2020). It plays a crucial role in food acquisition, processing, and climbing, as well as in various behavioral functions including biting, display, communication, territorial defense, vocalization, thermoregulation, and preening (McLelland, 1990; Klasing, 1999; Gelis, 2006; Kindersley, 2011; Venkatesan et al., 2015; Tattersall et al., 2017).

The morphological variation observed in bird beaks is a result of adaptations to diverse environmental conditions, dietary preferences, and foraging strategies. The shape and size of the avian beak significantly impact feeding and digestion processes, enabling species differentiation based on these morphological characteristics (Rossi et al., 2005; Gelis, 2006; Abumandour, 2014; Gupta et al., 2015; Tattersall et al., 2017; Navalón et al., 2019). The rate of wear and regrowth of the keratinized layers covering the beak influence its overall shape (Gelis, 2006; Klasing, 1999). Most of studies on the upper beak of a carnivorous bird are scanty. Therefore, this study aimed to provide the structural features of the upper beak of the little owl.

2. Material and methods

2.1 Birds: This study was conducted on 10 heads of normal, healthy, and adult little owls *(Athene noctua)* captured in Abu Rawash, Cairo Governorate, Egypt.

2.2 Gross morphological analysis: The heads were separated from the bodies and cleaned with running water. The oropharynx was exposed by cutting at the beak angle and examined using a magnifying lens.

2.3 Scanning electron microscopy preparation: Three little owl heads were prepared for scanning electron microscopy (SEM). The samples were washed in saline, fixed in a 2.5% paraformaldehyde and 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4) for 24 hours at 4°C, and post-fixed in 1% osmium tetroxide at room temperature. After dehydration in a graded ethanol series (50%, 70%, 80%, 90%, 95%, and 100%), the samples were critically point-dried in liquid carbon dioxide and gold-coated using an SPI-model Sputter coater. The samples were examined and photographed using a JSM-5500 LV scanning electron microscope (Joel, Japan) at 10-20 kV. The examination was conducted at the central laboratory of South Valley University, Egypt.

2.4 Histological investigation: Cross-sections of the upper beak of the little owl were washed and fixed in 10% neutral buffered formalin. Decalcification was performed using a solution of 100 ml formic acid, 10 ml formalin, and 800 ml distilled water, which was changed every three days. The extent of decalcification was examined by physical testing. After decalcification, the sections were re-fixed in 10% formalin for two days, washed in running tap water for 1-2 hours, and dehydrated in ascending grades of ethyl alcohol (70% overnight, 80%, 90% for 2 hours, 100% for 15 minutes). The sections were cleared in methyl benzoate and embedded in paraffin wax (paraffin-I for 3 hours, paraffin-II for 3 hours, and paraffin-III for 24 hours at 60°C). Sections of 5-7 μm thickness were cut, mounted on glass slides, and stained with Harris hematoxylin and eosin (H&E), Crossman's trichrome, periodic acid-Schiff (PAS), Alcian blue (AB), and combined AB + PAS stains.

3. Results

3.1 Gross anatomy: The upper beak of the little owl exhibited a rigid, triangular morphology with a broad base and a sharply pointed, strongly curved tip. It was longer than the lower beak, extending beyond its tip to form a hook-like structure. The upper beak had three surfaces: two dorsolateral and one ventral surface. The dorsolateral surface presented an elongated nostril at the base, which was oval-shaped, devoid of a horny covering, and vertically oriented. The upper beak was strongly convex dorsally. In contrast, the ventral surface was generally concave, with a particularly strong concavity at the tip. The lateral edges of the upper beak were smooth, thin, and sharp (Fig. 1 a).

Morphometric measurements showed that the width of the upper beak increased caudally. Additionally, the height of the upper beak increased caudally, reaching its maximum at the angle of the mouth. The beak's height increased significantly caudally from the tip to the nostril level.

3.2 Scanning electron microscopy: Scanning electron microscopy revealed that the tip of the little owl's upper beak was pointed and covered with numerous irregular keratinized flakes. Similar flakes were also observed on the lateral edges of the upper beak (Fig. 1b).

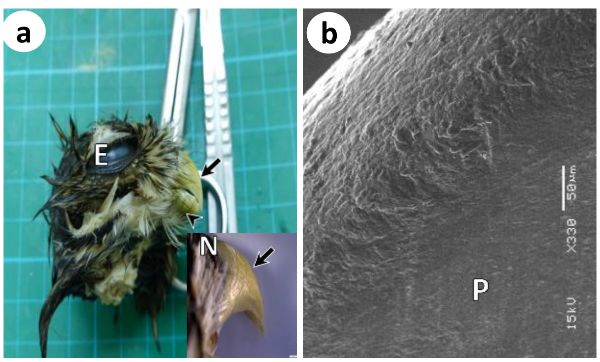

Fig. 1: (a) Gross micrographs of the upper beak and palate of the little owl showing the upper beak (arrowhead), nostrils (N), eye (E). (b) Scanning electron micrographs of the upper beak showing numerous irregular keratinized flakes on the edge of the upper beak.

3.3 Light microscopy: Light microscopic examination revealed that the upper beak comprised a bony foundation enveloped by dermal and epidermal layers of variable thickness. The bony basis was composed of premaxillary bones in the rostral and lateral regions. It was compact, consisting of numerous bony trabeculae surrounded by a thick outer bony shell and separated by small bone marrow spaces. The premaxillary bone was covered by a keratinized stratified squamous epithelium comprising multiple layers:

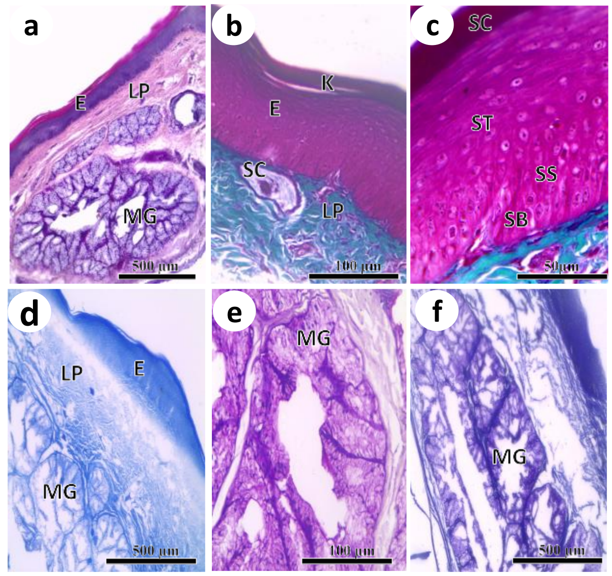

Fig. 2: Photomicrographs of cross sections of the upper beak (a, b, c, d, e, f) of the little owl showing keratinized stratified squamous epithelium (E), keratin layer (K), stratum basal (SB), stratum spinosum (SS), stratum transitivum (ST), stratum corneum (SC), lamina propria (LP), sensory corpuscles (SC), and maxillary glands (MG) .(a) HE stain, (b, c) Crossman's trichome stain, (d) AB stain (e) PAS stain, and (f) Combined AB/PAS stain.

the stratum basale, stratum spinosum, stratum transitivum, and stratum corneum. The stratum basale consisted of a single layer of basal cells adjacent to the basement membrane. The stratum spinosum was composed of multiple layers of polyhedral cells, typically three to four layers. The stratum transitivum consisted of several layers of flattened cells with flattened nuclei. The outermost layer, the stratum corneum, was composed of keratinized squamous cells (Figs. 2a, 2b, 2c). The submucosa formed upward projections into the mucosa known as dermal papillae. The dermis consisted of dense irregular connective tissue containing blood vessels and nerves. The dermis was rich in small capillaries and sensory corpuscles, primarily Herbst corpuscles. These corpuscles were located immediately beneath the epidermis, exhibiting rounded and elongated shapes. They were composed of central axons and sensory cells surrounded by a network of fine collagen fibers (Figs. 2a, 2b). The submucosa the upper beak contained numerous lobules of maxillary salivary glands. They showed positive PAS reaction and positive AB (Figs. 2d, 2e, 2f).

4. Discussion

The upper beak of the little owl, with its wide base and strongly curved, sharp-pointed tip, is similar to that found in carnivorous birds such as the great Indian horned owl, Eurasian hobby, falcon, and sea eagle (Ladyguin, 2000, Abumandour, 2014, Rajalakshmi et al., 2020, Preja et al., 2023).

Our results showed that the little owl, as a carnivorous bird, has a rigid hard beak with a curved sharp pointed tip. This agreed with that reported by (Pecsics et al., 2018) Who mentioned that little owls, feeding on smaller prey, have smaller beaks compared to other owl species. While smaller prey can be swallowed whole, larger prey is typically torn apart using the strong, curved beak and feet before being consumed. Furthermore (Navalón et al., 2019) reported that the curved beak is correlated with the consumption of specific food items, such as vertebrates and seeds, as an adaptation to higher stress.

Our Scanning electron microscopy investigation revealed that the tip and lateral edges of the upper beak were covered in numerous irregulars, keratinized flakes. These keratinized flakes, which form due to the shedding of epithelial cells during the manipulation of food, provide resistance to abrasion and help protect the beak from wear and tear (Seki et al., 2005; Sayed et al., 2014).

Ligh microscopical findings of the upper beak of the little owl showed that the beak's epithelium and the stratum corneum were significantly thick. The level of keratinization in bird beaks varies depending on their specific feeding habits (King and McLelland, 1984) and the degree of mechanical stress exerted by food items (Nickel et al., 1977; McLelland, 1979). The beak fulfills a diverse range of functions beyond mere feeding, encompassing drinking, nest material collection, defensive and offensive behaviors, preening, courtship rituals, display, and climbing (O'Malley, 2005; Gelis, 2006; Trivedi and Soni, 2013).

5. Conclusions

The present study investigated the anatomical features of the upper beak of the little owl, revealing various morphological characteristics. The upper beak of the little owl is curved, with a pointed tip and numerous irregular keratinized flakes covering both the tip and lateral edges. It is composed of a bony basis covered by dermal and epidermal layers.

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