Anatomical and Surface Electron Microscopic Investigation of the Tongue and Laryngeal Prominence in the Red-Eyed Turtle Dove (Streptopelia semitorquata, Rüppel 1837)

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Anatomical and Surface Electron Microscopic Investigation of the Tongue and Laryngeal Prominence in the Red-Eyed Turtle Dove (*Streptopelia semitorquata*, Rüppel 1837)

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Abstract

This study investigated the morphology of tongue and laryngeal structures in red-eyed dove and related it to feeding habits. Samples were examined using gross anatomy, scanning electron microscopy (SEM), and histological techniques. The tongue conformed to the shape of the lower beak, and the apex with a pointed tip, body, and root were distinguished. A median grove was apparent, and a papillary crest with pointed triangular papillae separated the body from the root. The length of the tongue, its width (body), and the percentage of the length of the lower jaw occupied by the tongue were, on average, 12.5 mm, 2.3 mm, and 57.3%, respectively. SEM showed highly desquamated dorsal epithelium with pointed papillae-like projections. The papillary crest presented pointed papillae of about 20–22. Salivary openings were apparent in the root, surrounded by mucosal folds and blunt papillae. Laryngeal prominence was irregularly triangular shaped and elevated. The rim of the glottis showed glandular pinpointed marks. Typical cornified multilayered mucosal epithelium was present in the apex and decreased caudally. Hyaline entoglossal cartilage and adjoining connective tissue were present in the body and apex. Numerous tubuloalveolar glands were seen in the body and root. The rim of the glottis showed mucous intraepithelial glands. The rostral and caudal lingual glands indicated positive reaction to neutral and acidic mucins. This study demonstrated a morphofunctional relationship of the tongue of a red-eyed dove to its diet.

Key words: anatomical, laryngeal mound, lingual, red-eyed dove, scanning electron microscopy

Introduction

Birds have been a source of fascination and inspiration to people since the beginning of history (Alberton, 2012). Birds represent a class of vertebrates that possess some remarkable features, which have empowered them with the adaptations for diverse conditions of life including marine existence (Bhattacharyya, 1994). The anatomy and function of the avian tongue differ from amphibians, reptiles, and mammals. The external form of the tongue has generally become specialized as a nonflexible structure with a variable shape due to a relatively rigid mucosal covering (highly keratinized stratified squamous epithelium) and cartilaginous entoglossum in several species (Parchami & Dehkordi, 2011; Erdoğan & Iwasaki, 2014; Iwasaki et al., 2019). The shape and development of the avian tongue vary markedly according to feeding mechanisms (Erdoğan & Iwasaki, 2014). The insectivorous and humming birds (*kolibris*) possess a very long and protrusible tongue and that of psittacines (gray parrot and cockatoo) is remarkably muscular, while the subfamily Loriinae (colorful parrots) show a brush-like apex for collecting nectar. In several avian species especially in the order Galliformes, the tongue is pointed apically and broad at its base with scant or absence of muscle (König et al., 2016). In general, it appears that stratification and keratinization of the lingual epithelium have been the most remarkable changes during the evolutionary adaptation of vertebrates from wet to dry ecosystems (Iwasaki, 2002). Therefore, in addition to the shape and length of the lower beak, the morphology of the avian tongue shows species-specific relationship necessary for acquisition, filtering, capturing, manipulation, and swallowing of foods. It is also related to the lifestyles exhibited in different environments (Bhattacharyya, 1994; Emura et al., 2008).

Some studies relating to the tongue and other oropharyngeal and musculoskeletal structures have been evaluated in several species of the family Columbidae such as laughing dove *Streptopelia senegalensis* (Al-Nefeiy, 2015; Farouk & Hassan, 2015; Abumandour & El-Bakary, 2019), rock pigeon *Columba livia dhakhlae* (Abumandour et al., 2021*a*), Indian collared dove *Streptopelia decaocto* (Trivedi, 2020), domestic pigeon *Columba livia domestica* (Parchami & Dehkordi, 2011), rock dove *Patagonias livia* (Al-Nefeiy, 2017), common pigeon, and imperial pigeons (Zweers, 1982; Bhattacharyya, 1980, 1990). In addition, El-Mansi et al. (2021) recently investigated the anatomy of the tongue and oropharynx of the Eurasian collared dove (*S. decaocto*) and their findings showed distinct adaptation for their dietary niche. There are also investigations on the lingual structures in other noncolumbid birds that have similar feeding preferences to red-eyed dove including quail (Parchami et al., 2010), chicken and quail (Iwasaki & Kobayashi, 1986; Abumandour et al., 2021*b*),

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house sparrow (Abumandour, 2018), chukar partridge (Erdoğan et al., 2012*b*; Sağsöz et al., 2013), European magpie and common raven (Erdoğan & Alan, 2012), zebra finch, and common starling (Dehkordi et al., 2010; Taha & Al-Duleemy, 2020). These species showed some similarities in the spread of their mechanical papillae, presence of papillary crest, and evidence of large conical papillae near the glottis. But they showed differences in microstructures of the tongue, the number of mechanical papillae, gustatory papillae, and the presence of salivary glands that support their feeding habits.

Generally, there have been extensive investigations of the tongue anatomy in several orders of birds such as Anseriformes, Piciformes, Apodiformes, Passeriformes, Galliformes, Falconiformes, Struthioniformes, Gruiformes, and Sphenisciformes, among others (Ewald & William, 1982; King & McLelland, 1984; Homberger & Meyers, 1989; Kobayashi et al., 1998; Liman et al., 2001; Samar et al., 2002; Downs, 2004; Jackowiak & Ludwig, 2008; Crole & Soley, 2009; Emura et al., 2009*b*; Pasand et al., 2010; Jackowiak et al., 2011; Erdogan et al. 2012*a*; Elyasi & Goodarzi, 2021, Bassuoni et al., 2022). Many of these studies were performed using gross anatomy, light microscopy, scanning and transmission electron microscopy. These studies have been extensively reviewed in comparative terms in relation to the shape of tongue, papillae distribution, surface features of epithelial layers, lingual glands, taste buds, and other movable musculoskeletal elements that help in avian feeding (Erdoğan & Iwasaki, 2014). Some authors described the microstructures of two types of keratinized epithelium as orthokeratinized (cell nucleus is absent in the keratinized layer) and parakeratinized (flattened and condensed nucleus, which is present in the cytoplasm until release by exfoliation); these later microstructural descriptions were in the white-tailed eagle (Jackowiak & Godynicki, 2005), domestic goose (Jackowiak et al., 2011), and Middendorff's bean goose (Iwasaki et al., 1997). The present study is of phylogenic importance and also will add to the volume of knowledge on morphological differences relative to feeding adaptations of birds.

There are over 16 species of the genus *Streptopelia*, which are commonly referred to as "turtle doves," slender, relatively long-tailed, gray, or brown pigeons (Godwin, 1983). The presence of a neck or collar patterns persists as the most salient external plumage characteristics. The turtle doves are native to temperate and tropical regions of Europe, Asia, and Africa (Slabbekoorn et al., 1999). The red-eyed turtle dove *Streptopelia semitorquata* is a species of birds in the family Columbidae, which includes pigeon and doves. It occurs across sub-Saharan Africa, including Southern Africa and West Africa (Nigeria), and is well adapted to living with humans. In Nigeria, it has been reported to have deleterious effects on cereals such as cowpea and soybean (Akande, 1982). The diets of the red-eyed dove in the wild rainforests and urban environment include grains, seeds, nuts, berries, fruits, flowers, grasses, and, occasionally insects, snails, and earthworms. This bird is not under any conservation list in Nigeria and not in the Red List of Threatened Species according to International Union for the Conservation of Nature, IUCN (Bird Life International, 2022). There is currently an attempt to use domestic pigeons and wild doves as experimental model to study the parasite–host relationship and investigate the evolution of vertebrate and bird species (Mineo et al., 2009; Shapiro & Domyan, 2013). The present study aimed at providing information on the anatomy of the lingual and laryngeal apparatus of the red-eyed dove. The results will be compared with previous studies in other birds with the same or different feeding diets and habits. The study may provide useful information about food digestion in relation to diet for the poultry feed and drug manufacturers.

Materials and Methods

Experimental Animals

A total of nine adult red-eyed turtle doves (five males and four females) were used in this study. The average weight of the dove was 170.9 g (sex differences were not considered in this study). Five of them were obtained from natural and accidental mortalities in the rural forests and urban areas of Nsukka, Southeastern Nigeria. Four birds were obtained from farmers who captured them on farmland. They were clinically observed without oropharyngeal injuries or abnormalities. Freshly dead birds (accident) were decapitated, and the head was preserved in 10% neutral-buffered formalin (NBF) solution for gross and histological study. The four birds captured alive by farmers were taken into the laboratory in bird cages. They were sacrificed by decapitation with well-maintained sharp blade according to the guidelines of Animal and Ethics Committee of the University of Nigeria, Nsukka, Southeastern, Nigeria. The entire animal handling procedure is in accordance with the Guide for the Care and Use of Laboratory Animals of Institute for Laboratory Animal Research of National Research Council (2011).

Gross and Microscopic Study

Five heads of the bird samples (three males and two females) were fixed in 10% NBF following death, and the oral cavity was dissected for gross observations and morphometric measurements. Thereafter, the tongues and the laryngo-glottal structures were dissected out. The tongue was cut into three parts, namely apex, body, and root, and with the laryngo-glottal (laryngeal prominence) structures were immersed in the same fixative for adequate fixation. Fixed samples were processed routinely following standard procedures (Bancroft and Gamble, 2008). They were cleared in xylene and embedded in paraffin wax. Tissue blocks were cut at 5–6 μm and stained with Harris hematoxylin and eosin (H&E) following routine histological procedures. In addition, the sections were also stained with periodic acid-Schiff (PAS) and Alcian Blue (AB, pH 2.5) to identify neutral and acidic mucins. The sections were mounted on glass slides with Euthanal® (Sigma-Aldrich, Darmstadt, Germany). Gross photographs were obtained with digital iPad camera (Air iPad 2). Photomicrographs were obtained from mounted sections using an AxioCamERC5S microscope (Zeiss, Germany). For histometry of some tissue components of the tongue, randomly selected H&E sections of the tongue (about 30 slides) were measured after standard sampling. The thickness of the tissue components of the apex and body of the tongue were ascertained (40× magnification) using ocular reticule calibrated with stage micrometer gauge and results were shown (Table 2).

Scanning Electron Microscopy

Whole fresh tongues of four birds (two males and two females) each made up of apex, body, root, and laryngeal entrance were fixed immediately in 2.5% glutaraldehyde with phosphate buffer (pH 7.3 at 40°C) for surface microstructural analysis

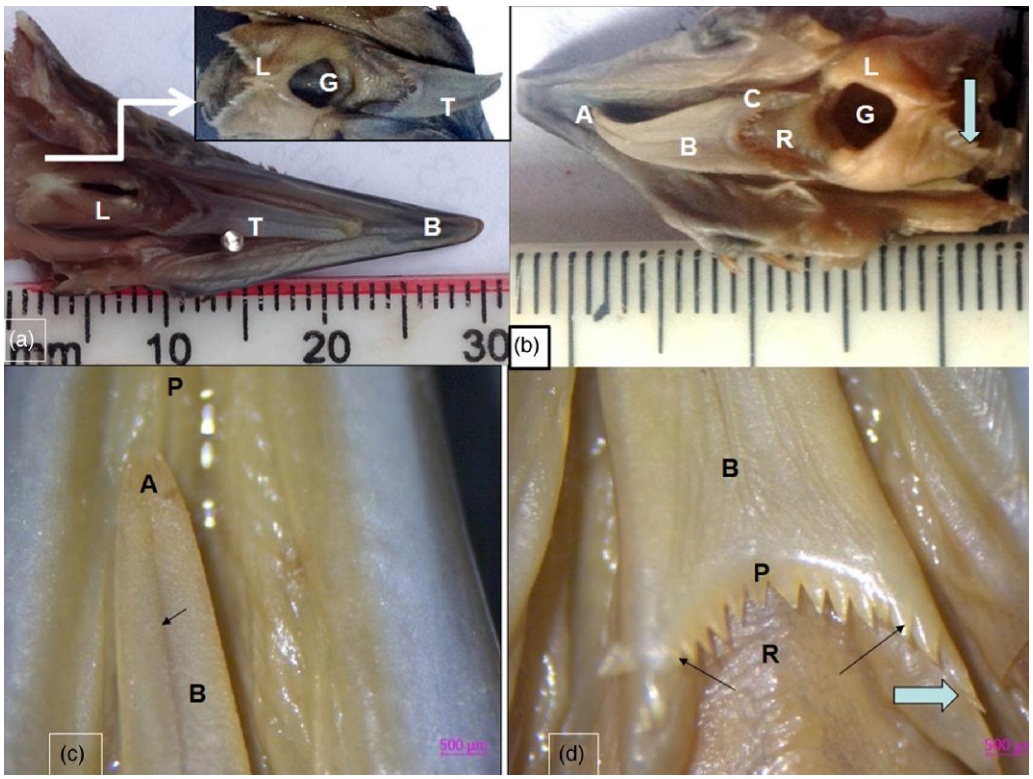

Fig. 1. (**a**) Gross photographs of the elongated triangular tongue by dissection: tongue (T), beak (B), and laryngeal prominence (L). Bar = 1 cm. (**b**) Gross photo showing parts of the tongue: apex (A), body (B), root (R), laryngeal prominence (L), glottis (G), papillary crest (C), pharyngeal papillae (arrow), and glottis (arrow). Bar = 1 cm. (**c**) Stereomicrographs showing the body of the tongue (B) and pointed apex (A) lodged in the lower beak (P). Bar = 500 μm. (**d**) Stereomicrograph showing the caudal body of the tongue (B), papillary crest (P) with pointed papillae (arrows), and root of the tongue (R). Bar = 500 μm.

in the scanning electron microscope (SEM). Adequately fixed tissues were postfixed in 1% osmium tetraoxide (2 h) and subsequently rinsed in the same buffer solution. Specimens were dehydrated through graded series of ethanol concentration (60–100%). Following satisfactory rinsing with phosphate buffer, a chemical drying agent hexamethyldisilizane (HMDS, Sigma-Aldrich, St Louis, MO, USA) was used following the manufacturer's instructions (Igbokwe & Mbajiorgu, 2019; Schu et al., 2021). This is an alternative to critical point drying (CPD). Well-dried tissue specimens were attached to aluminum stubs and coated with sputtered gold–palladium (in an Emscope SC500). Finished samples were examined and photographed using a TESCAN VEGA3 SEM (Brno, Czech Republic) operated between 6 and 10 kV at the Spectra Unit, located at the University of Johannesburg, South Africa.

Results

Macroscopic Features

The tongue of the red-eyed dove demonstrated a short and narrow triangular-shaped structure with a sharply pointed tip. It conformed to the shape of the lower beak in which it was tightly held (Fig. 1a). The tongue was distinguished into apex (apex linguae), body (corpus linguae), and root (radix linguae) with a definite landmark between the body and root in which the papillary crest was situated. The laryngeal prominence was a prominent elevated compartment with a median narrow slit (glottis) that opened into the larynx (Fig. 1b). Other remarkable features were a shallow median longitudinal sulcus that extended along the apex and body, followed by a serrated-edge papillary crest formed of conical-shaped papillae located in the boundary between the body and the root. Further views, with a stereomicroscope, showed that the dorsal epithelium had a pinpointed raised surface, which resembled desquamated surface epithelial structures (Fig. 1c). The conical-shaped papillae of the V-shaped papillary crest were about 20–22 in number and directed caudally toward the root. They were of variable sizes; the smallest size was closer to the midline. In addition, single, robustly pointed conical papillae were situated at the caudolateral edge of the papillary crest and classified as giant papillae (Fig. 1d). Rows of broad-shaped pointed pharyngeal papillae were located caudal to the glottal opening and demarcated the pharyngeal cavity from the tubular esophagus. These backward-directed papillae were about 20–24 in number (pharyngeal papillae) on the lateral sides and about 18–20 as clusters in the median and sagittal position. The gross morphometric values related to the tongue and laryngeal entrance are presented in Table 1.

SEM Observations

The SEM study demonstrated that the tongue was elongated, pointed at the apex with a median groove, and a slightly twisted ventral tip showed a minutely developed lingual nail (Fig. 2a). The dorsal and lateral surfaces of the tongue in the apex and body showed numerous uneven, highly desquamated, cornified lingual epithelial cells. The dorsal surface assumed uneven forms in the caudal part of the body of the

Table 1. Gross Morphometric Values Related to the Tongue and Laryngeal Entrance of Red-eyed Dove

Weight of bird (g)	170.9 (165.5–172.4)
Total length of tongue (mm)	12.5 (11.2–13.4)
Width of tongue—apex (mm)	2.1 (1.9–2.3)
Width of tongue—body (mm)	2.3 (2.1–2.4)
Width of tongue—root (mm)	3.5 (3.1–3.6)
Length of laryngeal mound (mm)	7.2 (6.8–7.4)
Length of laryngeal opening—glottis (mm)	3.2 (2.9–3.2)
Diameter of laryngeal opening—glottis (mm)	3.1 (2.7–3.2)
Diameter of laryngeal mound (mm)	2.2 (1.9–2.3)
Length of lower jaw (mm)	21.4 (21.1–21.6)
Percentage length of lower jaw occupied by tongue (%)	57.3 (56.2–57.8)

Table 2. Histometric Measurements Relating to the Tongue at the Apex and Body of Red-eyed Dove

Layer of Tissue Measured (Thickness)	Apex of the Tongue (μm)	Body of the Tongue (μm)
Keratin layer (dorsal)	75.2 (72.6–77.4)	—
Epithelium (dorsal)	199.1 (197.2–200.3)	143.4 (140.8–145.2)
Connective tissue (dorsal)	57.1 (55.3–58.5)	106.2 (105.3–108.4)
Lingual glands	—	65.2 (63.2–67.4)
Entoglossum (cartilage)	123.4 (122.1–124.9)	280.2 (278.1–283.3)
Connective tissue (ventral)	81.8 (80.4–82.3)	98.9 (97.2–99.7)
Epithelium (ventral)	105.3 (103.3–106.4)	56.2 (55.3–57.3)
Keratin layer (ventral)	80.5 (78.1–82.3)	18.5 (17.4–19.1)

tongue (Figs. 2b, 2c). In addition, the robust V-shaped papillary crest further clarified that the pointed papillae (about 20–22 in number) were triangular in shape (Fig. 2d). The dorsal surface showed microridge-like folds and was devoid of papillae. In addition, the root demonstrated preponderant small round openings of the lingual salivary glands that spread toward boundary of the elevated laryngeal mound (Figs. 2e, 2f, and insert). Under SEM, the laryngeal mound appeared as a highly elevated irregular triangular-shaped area. The inlet of the larynx (glottis) was an elongated narrow slit bounded by the arytenoid cartilage and was extended caudally by a laryngeal fissure (Fig. 3a). The laryngeal prominence was devoid of papillae at the rostral aspect, but behind the glottic opening, it was bordered by abundant conical papillae (pharyngeal papillae) arranged in rows on the lateral sides and in clusters of larger sizes on the median and sagittal position behind the caudal laryngeal fissure (Fig. 3b); they were directed caudally toward the root. The pharyngeal papillae delineated the pharyngeal floor from the entrance into the esophagus. Specifically, it was comprised of papillae on both lateral aspects of the laryngeal prominence (about 20–24 in number) and clusters of larger papillae (about 18–20 in number) in the median and sagittal position of the caudal entrance of the glottis near the laryngeal fissure. Some of these papillae appeared to have bifurcated tips (Fig. 3c). Caudal laryngeal prominence (with a laryngeal fissure) presented some openings of the laryngeal salivary glands as in the root (Figs. 3d, 3e). In addition, moderately developed mucosal folds bordered the caudal part of the glottis.

Histological and Histochemical Features of the Tongue

The histometric measurements relating to the tongue at the apex and body are presented in Table 2. Generally, the tongue on its dorsal and ventral surfaces showed variations in the histological layers that comprised a cornified multilayered mucosal epithelium and lamina propria of mucosa, which consisted of a thin layer of loose connective tissue with embedded lingual glands. There was no submucosa. In addition, the tongue was supported by the sagittal entoglossal cartilage structure and supported by a ventrally layer of longitudinal striated muscles. There were slight regional differences in thickness and composition of the epithelium and cartilaginous entoglossum in the apex, body, and root and in the laryngeal prominence (Figs. 4a–4f, 5a–5f). Specifically, in the tip and apex of the tongue, the dorsal and ventral surface epithelium was cornified multilayered mucosal epithelium and the lingual nail on the ventral surface was not distinctly developed. However, the ventral and lateral epithelial surfaces of the apex and body were considerably reduced in thickness (Figs. 4a–4c). Also in the apex, the lamina propria underneath the epithelium was comprised predominantly of loose connective tissue with copious blood vessels and skeletal elements, but was devoid of salivary glands. In the caudal aspect of the apex, a central hyaline cartilaginous rod (entoglossum) surrounded by a distinct perichondrium was apparent (Fig. 4d). There was also a gradual reduction in the thickness of the cornified mucosal epithelium toward the root of the tongue. The dorsal epithelium was made of multilayered cells of about three to four layers of polyhedral cells covered by few layers of exfoliated cornified epithelial cells especially at the apex and body. The dorsal epithelial layers typically showed basal layer (with cylindrical shape oval-shaped nuclei), intermediate layer with polytonally shaped cells, and cornified layer (flattened nucleated cells and somewhat elongated in shape) similar to parakeratinized epithelium recognized previously by some researchers (Fig. 4e). The basal layer of the dorsal epithelium was clearly defined. But the epithelium of the ventral surface showed remarkable reduction of the cellular layers and the nuclei were absent, resembling an orthokeratinized layer. These layers (three to four) of cells in the dorsal surface were covered superficially by spiky desquamated (exfoliated) epithelial cells on the dorsal surface (Figs. 4f, 5a, 5b). The entoglossum extended from the root through the body to the apex of the tongue (caudal aspect of the apex) and therefore occupied the entire of the body of the tongue. It was comprised of several chondrocytes lodged within their lacunae. The ventral surface of the apex demonstrated profound reduction in the thickness of the histological features as in the dorsal surface (Figs. 5c, 5d). In the body of the tongue, the general histoarchitecture was maintained with a few differences related to epithelia of the dorsal surface, lamina propria with the presence of glandular structures, entoglossum, connective, and epithelia of the ventral surface with its keratin layer. There was a gradual decrease in the mucosal cornified multilayered epithelial thickness of the dorsal and ventral surfaces. Copious glandular lobules were apparent (Fig. 5e). The lingual glands were of branched tubuloalveolar type and the acini of secretory units were located in the body lateral to the barrel of the cartilaginous hyaline plate. These glands were encased by thin connective tissue capsule, with septa that demarcated them into lobules (Fig. 5f). The salivary

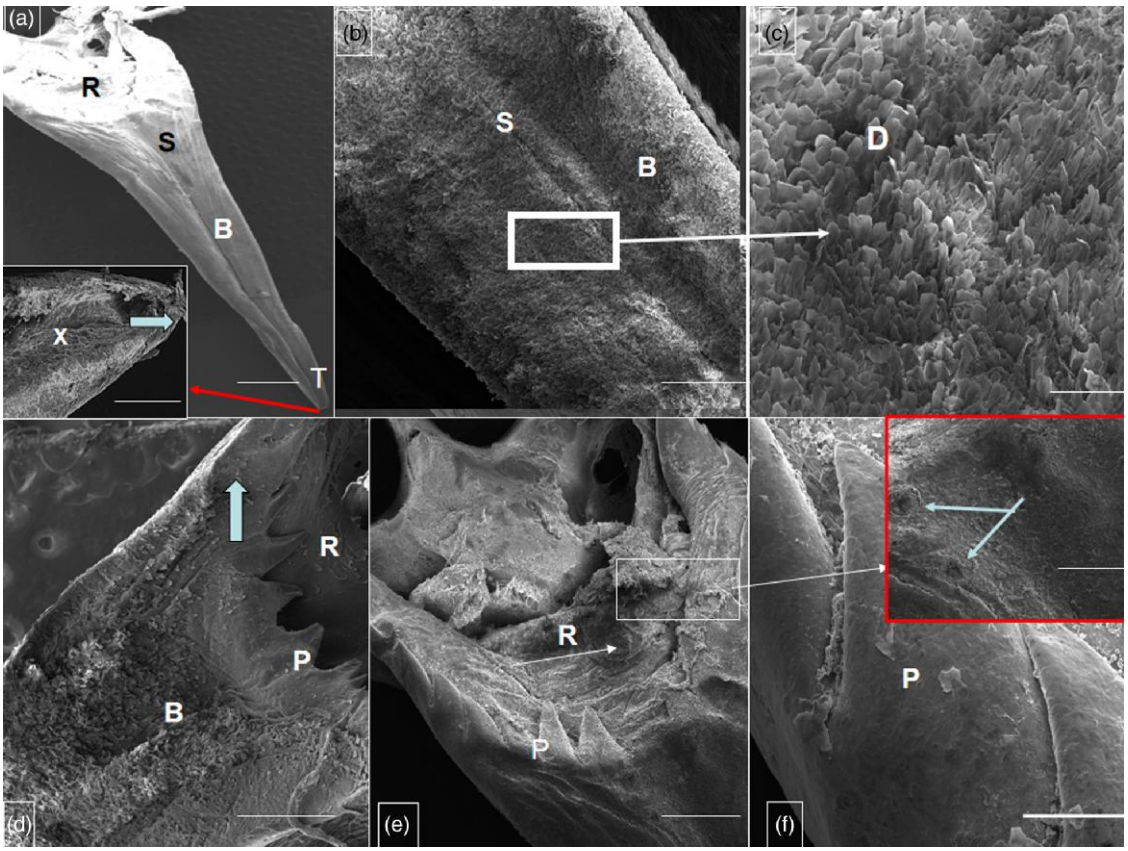

Fig. 2. (**a**) SEM micrograph showing a distinguishable part of the tongue and its pointed shape: root (R), body (B), median groove (S), and tip of the apex (T) with a pointed minute lingual nail (arrow). Bar = 500 μm. Insert: SEM photo of the apex (X) showing a minute lingual nail at the ventral surface (arrow). Bar scale = 200 μm **(b)** The dorsal and lateral surfaces with filiform-shaped desquamated projections (rectangle box) in the body (B) and median groove (S). Bar = 500 μm. (**c**) The scaly desquamated (exfoliation) projections in higher magnification (D). Bar = 100 μm. (**d**) The desquamated surface of the caudal portion of the body (B), the robust V-shaped papillary crest (P), and the scale-like root beyond (R). Bar = 500 μm. (**e,f** and insert) The root of the tongue (R) with openings of salivary glands (arrow), the papillae of the crest (P), and higher magnification of the salivary openings are indicated in the insert (arrows). Bar = 1 mm (**e**), 100 μm (**f**), and 200 μm.

lobules occupied a dorsolateral position in relation to the entoglossum.

Further transverse sections demonstrated that the entoglossum was transformed into a robust laterally spread bilobed structure in the caudal extremity of the body (Fig. 6a). A few bundles of transverse and longitudinal skeletal muscle were observed. The blood and lymphoid structures increased in this region of the tongue within the lamina propria of the ventral surface.

In the boundary between the body and root, a papillary crest with numerous large pointed conical-shaped papillae was detected (Fig. 6b). It possessed a nonkeratinized stratified squamous epithelium and their tips pointed backward toward the root. Underneath the surface epithelium was a typical connective tissue core bent caudally. The lamina propria also displayed caudal salivary gland acini (Fig. 6c).

In the region of the root of the tongue, the dorsal was nonkeratinized stratified squamous epithelium, while the ventral surface was attached to the base of the tongue. In addition, skeletal muscle fibers were apparent between the entoglossal cartilage and ventral surface. The connective tissue of the lamina propria spread deeply into the epithelium of the tongue and formed narrow strands of connective tissue papillae that spread toward the overlying surface epithelium. Taste buds were not present. The submucosal layer contained numerous simple tubuloalveolar salivary glands. They appeared as separate lobules covered by connective tissue capsule and with glandular ducts, which opened to the surface. Their lobular secretory units resemble the rostral lingual glands of the body. The superficial salivary units opened on the dorsal surface, while the deeper collections of salivary secretory units appeared to open indirectly through excretory ducts into the dorsal surface (Figs. 6c, 5d).

The laryngeal prominence displayed nonkeratinized stratified squamous epithelium similar to the root of the tongue, and this continued to the edges of the glottis (Figs. 6e, 6f, 7a). However, the rim of the glottis (laryngeal opening) showed a ciliated pseudostratified columnar epithelium (respiratory epithelium). There were intraepithelial mucous secretory glands, which opened into the pharyngeal cavity. The mucous glands were lined by a single layer of low columnar mucous secreting cells with flattened basally located nuclei and foamy vacuolated cytoplasm.

Histochemical staining used PAS to demonstrate neutral mucins and AB (pH 2.5) to display acidic mucins. These reactions showed that the lingual salivary glands located in the body (rostral lingual glands) and in the root (caudal lingual glands) were moderately PAS positive and AB positive in their contents (Figs. 7b, 7c, 7e). Similarly, the salivary glands in the laryngeal mound (cricoarytenoid glands) showed strong positive reaction to PAS and AB. In addition, the intraepithelial mucous glands of the epithelial rim of the laryngeal opening

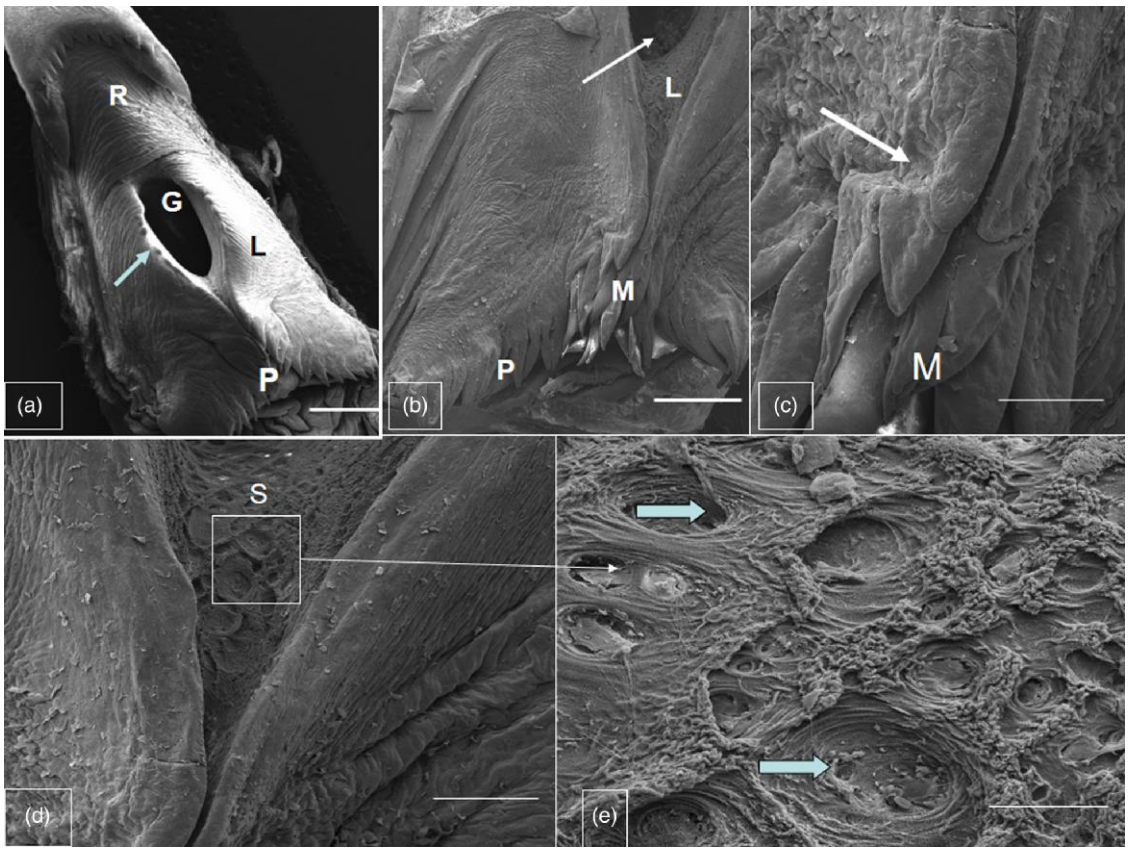

Fig. 3. (**a**) SEM micrographs showing the highly elevated laryngeal prominence (L), the glottis (G), and elevated glandular structures on the rim (arrow), root (R), and pharyngeal papillae (P). Bar = 5 mm. (**b**) The glottis (arrow) and laryngeal sulcus (L), robust pharyngeal papillae (P) in the lateral aspect, and clusters of pharyngeal papillae on the median and sagittal aspects of the laryngeal sulcus (M). Bar = 1 mm. (**c**) Higher magnification of the papillae (M) on median aspect and bifurcated papillae (arrow). Bar = 200 μm. (**d**) The glandular structures within the laryngeal sulcus (S). Bar = 500 μm. (**e**) Openings of the laryngeal glandular structures in the sulcus (arrows). Bar = 50 μm.

(glottis) also showed moderate positive reaction to PAS and AB stains.

Discussion

The current study evaluated the morphology of tongue and laryngeal structures in red-eyed dove and compared it to its feeding habits and environment. Generally, there have been extensive investigations of the tongue anatomy in several orders of birds that have been extensively reviewed and experimental data provided (King & McLelland, 1984; Iwasaki, 2002; Erdoğan & Iwasaki, 2014). However, the tongue of the red-eyed dove has not been examined previously. This study provided the missing anatomical information on this important avian species relative to diet and dwelling environment.

The avian tongue and laryngeal structures play very crucial functions in birds such as capturing, filtering, manipulating, swallowing, taste perception of food, and in vocalization (Mason & Clark, 2000; Erdoğan & Iwasaki, 2014). Previous studies across many species in several orders of birds have shown that there are some modifications in the tongue and oropharynx that relate to feeding habits and environmental conditions in the species so far examined (King & McLelland, 1984; Erdoğan & Iwasaki, 2014). The specialization of the feeding apparatus, especially the tongue in conjunction with the adaptive mechanism of feeding, enables avian species to survive and adapt to environmental changes. This is important because birds require enormous energy to sustain their metabolic activity during both flying and resting periods.

In this report, minor variations were seen in the dimensions of the tongue, papillae distribution, and other features of the epithelial layers and glands of the red-eyed dove in comparison with other members of the Columbidae family such as the laughing dove, rock dove, and pigeons, which feed primarily on seeds, fruits, and plants. On the contrary, remarkable differences existed between the red-eyed dove and other birds, which are granivorous, omnivorous, and carnivorous in feeding preferences, such as common pheasants, the Japanese pygmy woodpecker, guinea fowl, the chukar partridge, domestic chicken, common raven, nut cracker, common quail, common kestrel, Hume's tawny owl, and house sparrow (Emura et al., 2009*b*; Jackowiak et al., 2010; Parchami et al., 2010; Erdoğan et al., 2012*b*; Erdoğan & Alan, 2012; Abumandour & El-Bakary, 2017; Abumandour, 2018; Ertaş & Erdoğan, 2019; Ilgün et al., 2020; Elyasi & Goodarzi, 2021), respectively. In addition, the current study that determined the size of the tongue in the red-eyed dove to be about 12.5 mm in length and width (body) of about 2.3 mm (body weight: 170.95 g) differed from that of laughing dove (*S. senegalensis*), which measured about 21 mm in length and 5 mm in width at the caudal aspect of the root and the body weight was about 81.4 g (Al-Nefeiy, 2015). Trivedi (2020) in the study of some Indian doves obtained the mean values of 10.63 mm (length) and 3.88 mm (width) in the tongue of Eurasian collared dove *S. decaocto*. Also values of 9.88 mm

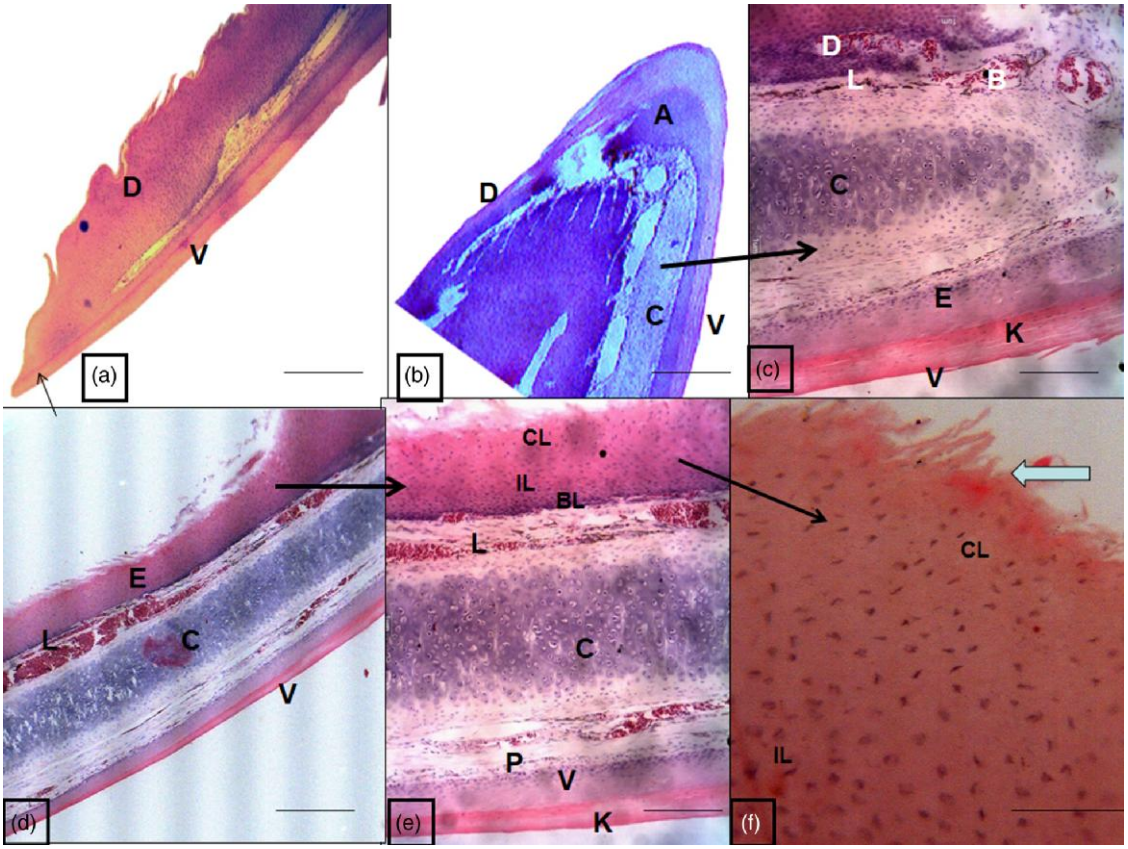

Fig. 4. (**a**) The photomicrograph of the histological section of the apex of the tongue with dorsal surface (D), which is a cornified multilayered mucosal epithelium (parakeratinized epithelium), and ventral surface (V) with thin cornified epithelium (orthokeratinized epithelium); the apex is keratinized on both surfaces (A) with a poorly formed lingual nail (arrow). Bar = 150 μm. (**b**) Photomicrograph of the apex of the tongue indicating the cornification of the dorsal (D) and ventral surfaces of the apex (V). Bar scale = 100 μm, H&E. (**c**) Photomicrograph of the apex of the tongue showing larger magnification that identified the dorsal epithelium (D), lamina propria connective tissue (L), blood vessels (B), entoglossum termination (C), ventral surface (V), ventral cornified epithelium (E), and keratin layer (K). Bar scale = 100 μm. (**d**) Photomicrograph showing the apex and initial part of the body with desquamated (exfoliated) dorsal epithelium (E), vascularized lamina propria (L), entoglossum (C), and gradual reduction of the keratin layer of the ventral surface (V). Bar scale = 100 μm. (**e**) Photomicrograph of higher magnification of (**b**), with desquamated surface of dorsal surface (E), lamina propria (L), entoglossal cartilage (C), lamina propria of ventral surface (P), ventral epithelium (V), cornified layer (CL), intermediate layer (IL), and basal layer (BL). Bar scale = 100 μm. (**f**) Photomicrograph of the surface desquamated/exfoliated dorsal epithelium (arrow) showing the cornified cellular layer (CL) and intermediate layer (IL). Bar scale = 60 μm, H&E.

(length) and 3.13 mm (width) were obtained for the laughing dove *Stigmatopelia senegalensis* in the same study, which did not differ remarkably from the present result. The tongue measured about 2.4 cm (length) in the nut cracker *Nucifraga caryocatactes* with body weight of 106–161 g (Jackowiak et al., 2010) and 15.97 mm (length) and 5.59 mm (width) in the chukar partridge *Alectoris chukar* with body weight of about 510–680 g (Erdoğan et al., 2012*b*). The salient differences in dimension of the tongue in correlation with the weight in the red-eyed dove and other birds may be due to dietary differences and genetic factors, and it appears to be species specific. The evolutionary pressures in different habitats have resulted in a variety of adaptations and allowed the birds to obtain food to survive. These adaptive features include a combination of the length and weight of the tongue, bill shape, and the body weight to match with the specific needs of each avian species.

In the current study, the tongue was not protrusible and is classified similarly among those tongues used for collecting food such as grains and seeds (Erdoğan & Iwasaki, 2014). The highly elongated triangular pointed tip of the tongue in both sexes conforms to the typical shape described in species of the order Columbiformes (Parchami & Dehkordi, 2011; Farouk & Hassan, 2015; Abumandour & El-Bakary, 2019; Abumandour & Kandyel, 2020; Mady, 2020). Three anatomical parts such as apex, body, and root were distinguished in the present report as in the previous reports on the Columbidae order. However, the tongue of the Egyptian laughing dove was described as round shaped (Abumandour & El-Bakary, 2019), which is similar to the description in goose, quail, and duck (Emura, 2009; Parchami et al., 2010; Jackowiak et al., 2011; Mohamed, 2019). Moreover, other varieties of shape of the tongue have been described in several families/orders such as elongated with shovel like or oval tip in families such as Falconiformes including white-tailed eagle, buzzard, and common kestrel (Jackowiak & Godynicki, 2005; Erdoğan et al., 2012*a*), elongated flat with rounded tip in Anseriformes (domestic goose) and Egyptian goose (Hassan et al., 2010; Jackowiak et al., 2011), and bifid or oval tip in Strigiformes (owls; Emura & Chen, 2008). The characteristics of varied shapes have been extensively reviewed and indicate adaptations for types of food eaten, mode of collection of food, swallowing mechanism, and method of manipulation in the oropharynx and other feeding habits (Erdoğan & Iwasaki, 2014).

The present findings included a distinct median groove that clearly divided the apex and body of the tongue into two

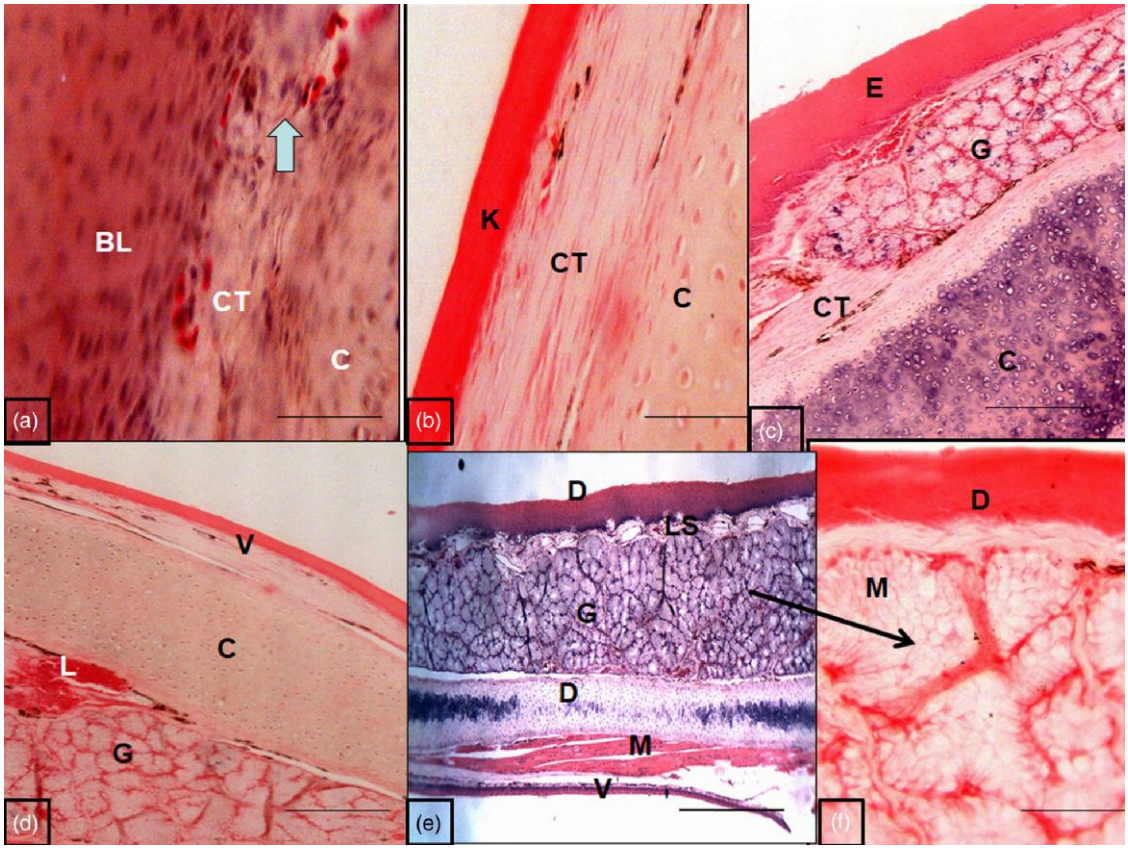

Fig. 5. (**a**) Photomicrograph of higher magnification of the basal layer (BL) of the dorsal surface of the multilayered mucosal epithelium, lamina propria connective tissue (CT) with vascular elements (arrow), and entoglossum (C). Bar scale = 60 μm, H&E. **(b)** Photomicrograph of the apex and initial part of the body (corpus linguae) showing the ventral surface of the tongue with cornification (K), lamina propria (CT), and entoglossum (C). Bar scale = 60 μm, H&E. (**c**) Photomicrograph of the body of the tongue showing components at this zone including the dorsal surface with poor or absence of a cornified layer (E), the lamina propria (CT) with glandular lobules (G) and blood vessels, and the abutting entoglossum (C). Bar scale = 100 μm, H&E. (**d**) Photomicrograph of the body showing the ventral surface orthokeratinized epithelium (V), connective tissue, cartilage (C), and glandular lobules (G). Bar scale = 100 μm, H&E. (**e**) Photomicrograph of a longitudinal section of the body close to the base of the tongue with various components: dorsal epithelium (D), lamina propria (LS), glands (G), entoglossum (D), muscle bundle support (M), and ventral surface (V). Bar scale = 100 μm, H&E. (**f**) Photomicrograph of the body of the tongue at high magnification showing mucous glandular lobules (M) and dorsal epithelium (D). Bar scale = 60 μm, H&E.

symmetrical halves. This feature is also seen from apex to body in the eagle, kestrel (Falconiformes), ducks, and goose in the order Anseriformes (Emura et al., 2008; Jackowiak et al., 2011), but absent in the Galliformes such as chicken (Iwasaki & Kobayashi, 1986; Homberger & Meyers, 1989). It is also absent in owls (Strigiformes), ostrich (Struthioniformes), and penguins (Sphenisciformes) as was previously reported (Erdoğan & Iwasaki, 2014). The presence of a median groove was postulated to indicate the ability of birds to deliver grains as whole packs into the esophagus (Alsanosy et al., 2021).

The present study did not encounter true lingual papillae, but some projections of highly desquamated superficial cells with sharp-pointed protrusions (processes) were visible under SEM and histological sections. Similar descriptions described the tongue of the laughing dove (Farouk & Hassan, 2015). Similar lingual elevations were described in the Egyptian laughing dove as numerous caudally directed filiform papillae in the apex and scale-like filiform papillae in the rostral part of the lingual body (Abumandour & El-Bakary, 2019). Furthermore, such desquamated cells and related projections have been described as carpet-like processes in some Galliformes (Homberger & Meyers, 1989) and acicular or needle-like processes in Passeriformes (Dehkordi et al., 2010; Jackowiak et al., 2010). Also, scale-like projections and thread-shaped protrusions were reported in Falconiformes (Jackowiak & Godynicki, 2005). In addition, desquamated cells were reported in owls and the oriental scops owl (Emura et al., 2009*a*) and they formed filiform processes and connective tissue cores of the epithelium. Moreover, such lingual structures were also described variously as filiform papillae on the dorsal surface of the body of the tongue in common pheasant and chicken (*Phasianus colchicus* and *Gallus domesticus*; Iwasaki & Kobayashi, 1986; Elyasi & Goodarzi, 2021), as short caudally directed scale-like filiform papillae in domestic pigeon (Parchami & Dehkordi, 2011; Abumandour & Kandyel, 2020), and as prominent protrusions of the deciduous epithelium on the caudal region of the tongue in quail, *Coturnix coturnix japonica* (Pourlis, 2014). The presence of highly desquamated/exfoliated cells in the present report may be due to variable abrasion patterns caused by the type of food consumed such as cowpea and soybean, as has been suggested for the laughing dove (Farouk & Hassan, 2015).

This study demonstrated a serrated papillary crest in the form of conical papillae in the boundary between the body and root. They were about 20–22 in number with single large one at the lateral edge of the V-shaped crest. Similar conical structures were described in other Columbiformes such as

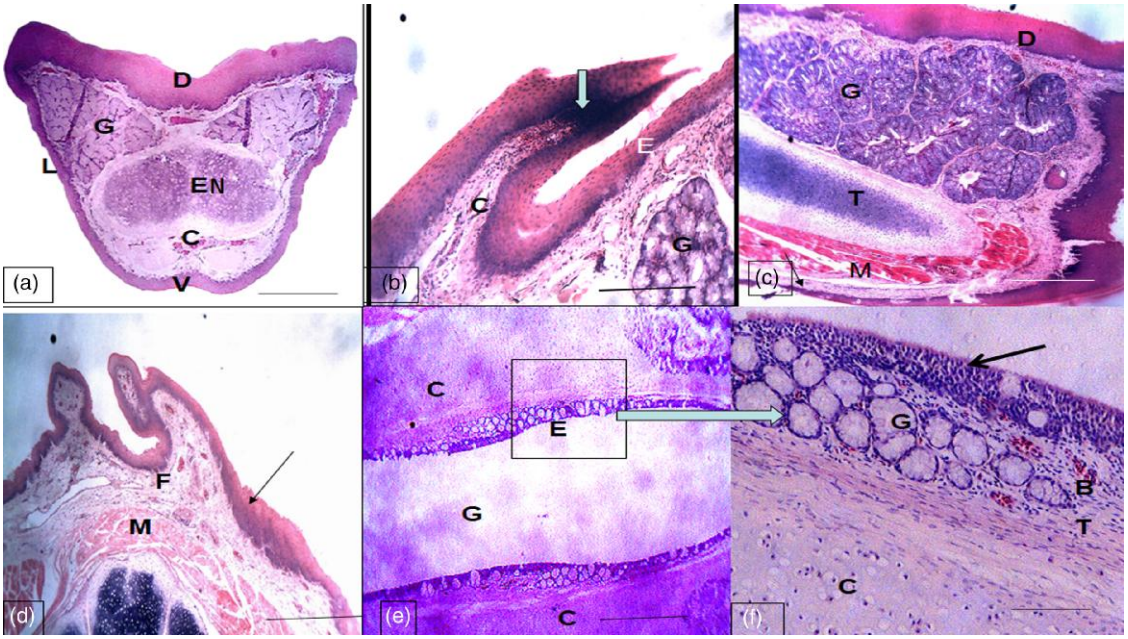

Fig. 6. (**a**) Photomicrograph of the histological section of the body of the tongue further indicating the dorsum of the tongue (D), ventral surface of the tongue (V), position of lingual glands (G) in relation to the bilobed entoglossum (EN), the position of lingual glands (G) in relation to the bilobed entoglossum (EN) at this level, dorsal surface, lateral surface (L), ventral surface (V), and connective tissue core (C). Bar = 150 μm, H&E. (**b**) Photomicrograph of the papillary crest before the root showing pointed papillae (arrow) with connective tissue core (C), and under are salivary tubuloalveolar acini (G) and dorsal epithelium of the root (E). Bar = 100 μm, H&E. (**c**) Photomicrograph showing caudal salivary gland units (G) above the cartilaginous support of the tongue base (T) and muscle bundles (M). Bar scale = 100 μm, H&E. (**d**) Photomicrograph of the root showing highly folded epithelium (F) with nonkeratinized epithelium (arrow). Bar scale = 150 μm, H&E. (**e,f**) Photomicrograph of laryngeal prominence showing rim of glottis (G) with intraepithelial mucous glands (E) and supported by arytenoid cartilage (C), and the ciliated pseudostratified columnar epithelium (E) contained mucous glands (arrow), connective tissue (T), and blood vessels (B). Bar scale = 150 μm (**e**) and 100 μm (**f**), H&E.

laughing dove, rock dove, rock pigeon, and Eurasian collared dove (Abumandour & Kandyel, 2020). More so, this arrangement of papillary crest is well represented in the Galliformes, Passeriformes, and Falconiformes, but true papillary crest is absent in ratites (ostrich) and penguins as outlined in the excellent review data of Erdoğan & Iwasaki (2014) and was investigated specifically in ostrich (Guimarães et al., 2009) and penguins (Kobayashi et al., 1998). However, there is no relationship between presences of papillary crest with feeding habits (Erdoğan & Iwasaki, 2014). The papillary crest is present in many avian species and across many families and orders. It is also present in herbivorous, omnivorous, carnivorous, and filter-feeding birds. It appears that large conical papillae on the crest help to transfer ingested food materials toward the esophagus and prevent its regurgitation ((Erdoğan & Iwasaki, 2014).

Histologically, the dorsal and ventral surfaces of the apex and body of the tongue displayed a cornified mucosal epithelium, which decreased in thickness toward the root of the tongue, and the ventral cornified layer was thicker. The current work showed with light microscopy a dorsal mucosal surface comprised of a three-to-four-layered mucosal surface precisely described as parakeratinized epithelium and a ventral surface referred to as orthokeratinized with loss of nuclei in the goose, duck, and turkey (Skieresz-Szewczyk et al., 2014, 2022). However, further advanced studies of the tongue in dove are warranted to evaluate the detailed nature of the cornified multilayered form of the tongue using transmission electron microscopy and immunohistological staining techniques. Much earlier observations were noted in the pigeon (Abdel-Megeid et al., 2021), but in this species, the keratinized layer extended to the root. Also, the keratinized layer was thickest in the ventral aspect compared to the dorsal epithelium, as was previously reported in laughing dove (Farouk and Hassan, 2015) and domestic pigeon (Mady, 2020). It was reported that the common pigeon (Igwebuike et al., 2013) had nonkeratinized epithelium on the dorsal surface, while the ventral surface demonstrated a well-developed keratinization. It may be possible that the cornified layer was washed-off the dorsal epithelium of the domestic pigeon during processing. The keratinization/cornification of dorsal epithelium has been reported in most avian species so far investigated (Erdoğan & Iwasaki, 2014). The keratinized dorsal epithelium was commonly observed in herbivorous birds to support in manipulation of food (Iwasaki, 1992). It is worth noting that the demonstration of two types of cornified mucosal epithelium—parakeratinized and orthokeratinized epithelium—in the domestic duck (Skieresz-Szewczyk et al., 2014), turkey (Skieresz-Szewczyk et al., 2021), and domestic duck, domestic goose, and domestic turkey (Skieresz-Szewczyk et al., 2022) undoubtedly shows that a different form of cornification of the mucosal layer of the tongue is present in birds different from the previous typical descriptions in many avian species. On the extreme variation, a keratinized dorsal and ventral epithelium is absent in ratites such as ostrich (Pasand et al., 2010). It seems that a nonkeratinized dorsal epithelium is not important in food manipulation (Gusseklo, 2006). In many birds, the dorsal epithelium of the apex of the tongue is in contact with food and may be injured during feeding, hence the need for adequate keratinized/cornified multilayered epithelium layer (dorsal surface) for protection in the granivorous bird such as the red-eyed dove. The tongue and oral cavity of the dove are exposed to solid and dried grains and seeds during feeding.

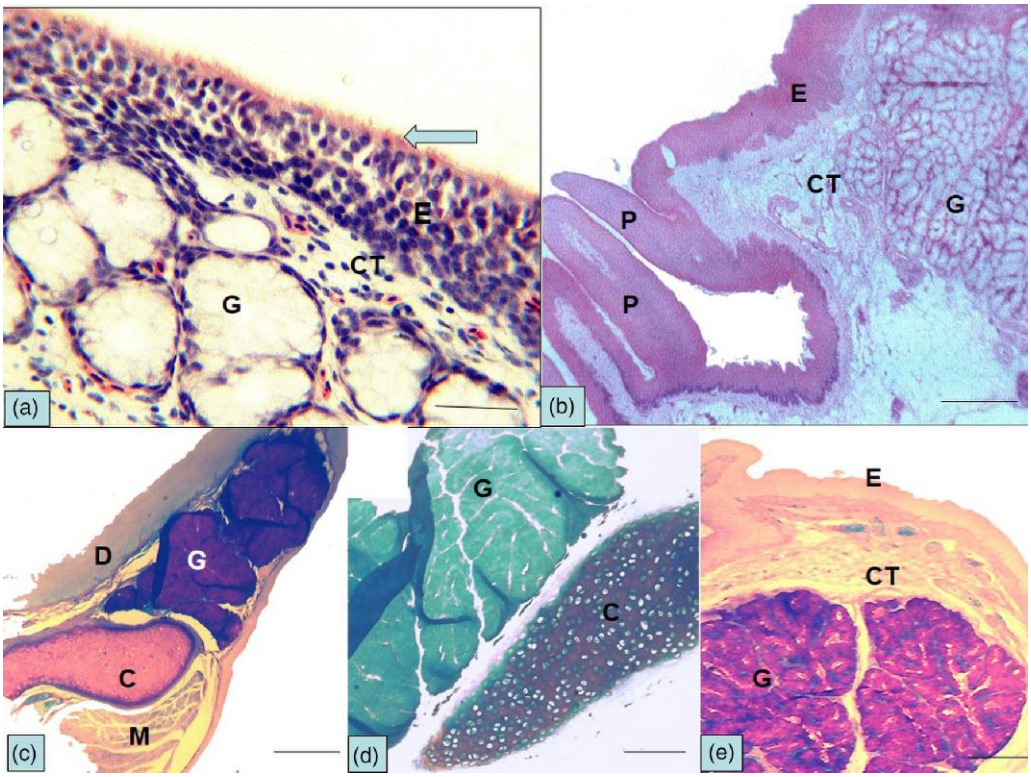

Fig. 7. (**a**) Photomicrograph of the higher magnification of the ciliated pseudostratified epithelium of the glottis (E) and ciliated surface (arrow), mucous glands, and connective tissue (CT). Bar scale = 60 μm, H&E. (**b**) Photomicrograph of the caudal laryngeal prominence showing epithelium (E), clusters of pharyngeal papillae (P), underneath connective tissue (CT), and glands (G). Bar scale = 100 μm, H&E. (**c**) Photomicrograph of the gland of the tongue body (B) showing a positive staining reaction to neutral mucins, dorsal epithelium (D), glands (G), skeletal muscles (M), and cartilage stump of the entoglossum (C). Bar = 100 μm, PAS. (**d**) Similar reactions with AB demonstrated acidic contents of the glands (G) and cartilage (C). Bar = 100 μm, AB. (**e**) Photomicrograph of the caudal salivary glands in the root showing a moderate positive reaction to neutral mucins (G), connective tissue (CT), and dorsal epithelium (E). Bar = 100 μm, PAS.

The lingual nail is a strong and hard keratinization/cornification of the ventral epithelium found at the tip of the apex in some birds. In birds such as nutcrackers, the lingual nail is a pair of dagger-like processes and is used in levering up, removing shells of seeds, and lifting grains into the median lingual sulcus for swallowing (Jackowiak et al., 2010). It was observed in minute form in the present report of orthokeratinized ventral surface epithelium of the red-eyed dove. Lingual nail was not reported in closely related laughing dove (Abumandour & El-Bakary, 2019), but El-Mansi et al. (2021) and Trivedi (2020) identified it in the Eurasian collared dove *S. decaocto* and laughing dove *S. senegalensis* similar to this report. The absence of this structure in some species of Columbiformes may be probably associated with genetic variability. The lingual nail has also been observed in some species of Columbiformes including rock pigeon (Abumandour & Kandyel, 2020), domestic pigeon (Mady, 2020), and common Egyptian pigeon (Abdel-Megeid et al., 2021). It is well developed in the apex and body of Anseriformes and present on the rostral tip of the apex in Galliformes, but absent in several orders of birds (Erdoğan & Iwasaki, 2014). The presence of well-developed lingual is seen in birds that are actively involved in grazing, pecking, and filter feeding as in the domestic ducks and goose (Skieresz-Szewczyk et al., 2014).

The entoglossum is part of the hyoid apparatus that is embedded in the tongue of birds (Homberger, 1986). In the present report, this highly cartilaginous rod was distinctively placed in the lingual body and stretched toward the tip of the tongue. It was housed by connective tissue with penetration of blood vessels (probably an evidence of early ossification). Similar placement of the entoglossum was reported in the pigeon (Abumandour & Kandyel, 2020) and laughing dove (Abumandour & El-Bakary, 2019). In addition, the same arrangement was noted in the Hume's tawny owl and common kestrel (Abumandour & El-Bakary, 2017). Serial transverse sections of the tongue at the caudal extremity of the body in the red-eyed dove showed that the entoglossum was transformed to a robust laterally spread bilobed structure—an observation that differs from the paired form reported in parrots and budgerigars, in which it is bifurcated rostrally in exceptional cases (King & McLelland, 1984). The entoglossum, therefore, contributes to the rigidity of the avian tongue. It has poorly developed intrinsic muscles and possesses thick epidermal epithelium (Tomlinson, 2000).

In addition to other remarkable lingual features, the lingual salivary glands and taste buds also represent important specialization of the avian tongue for lubrication of feed into the ball for easy swallowing and for taste perception (Iwasaki, 2002). In the present report, the SEM features demonstrated small openings of lingual salivary glands (caudal salivary glands). Histologically, some tubular alveolar glands were apparent in the lamina propria of the body and root in a dorsolateral position to the entoglossum. They were PAS and AB positive. Furthermore, the rim of the glottic opening of the laryngeal prominence showed intraepithelial mucous glands. However, several studies have demonstrated that the location and histological features of lingual salivary glands are species specific in birds. However, salivary glands in the

current study displayed similarities to other Columbiformes species such as the Eurasian collared dove (El-Mansi et al., 2021) and rock dove (Al-Nefeiy & Alahmary, 2015). The staining characteristics and mucin contents were also comparable to other avian species such as chukar partridge, quail, and chicken (Erdoğan et al., 2012*b*; Liman et al., 2001). Generally, gland location in many avian species is in the loose connective tissue of the lamina propria in the body and root of the tongue. It is characteristically simple, branched tubuloalveolar, or complex tubular in the Galliformes, ratites, and Columbiformes (Erdoğan & Iwasaki, 2014). The ovoid mucous secretory units in the root, body, and rim of the glottic opening demonstrated a moderate reaction for neutral and acidic mucins similar to the Eurasian collared dove (El-Mansi et al., 2021), chukar partridge (Erdoğan et al., 2012*b*), and quail (Liman et al., 2001). It shows that salivary glands have some genotypic convergence with regard to their types of food (Crole & Soley, 2009). It has been reported that mucous secretions of several mammals and birds lubricate the oral cavity, pharynx, and esophagus to facilitate the moistening and initiation of food digestion and deglutition (Iwasaki et al., 1997; Erdoğan & Alan, 2012). Other workers have suggested that mucous film offers a protective role against oral cavity desiccation and prevents undesirable activities of bacterial flora (Samar et al., 2002; Crole & Soley, 2009). Concerning salivary duct openings in the red-eyed dove, the results showed elliptical openings in the dorsal surface of the root. The glands were encased with connective tissue and opened on the surface through glandular ducts, as reported in several species across many families of birds (Erdoğan & Iwasaki, 2014). Also, similar openings were reported in the closely related domestic pigeon (Mady, 2020), rock pigeon (Abumandour & Kandyel, 2020), and Eurasian collared dove (El-Mansi et al., 2021).

The laryngeal prominence of the red-eyed dove under SEM showed a highly elevated irregular heart-shaped area that was devoid of papillae rostrally. But behind the glottic opening, it was bordered by conical papillae (pharyngeal) of variable sizes and arrangements, in addition to preponderant mucosal folds. Consistent with the present report, the laryngeal mound has been described in various shapes such as being irregular in the house sparrow (Abumandour, 2018) and European magpie (Erdoğan & Alan, 2012). But such papillae with variable numbers and arrangements were not seen in the southern lapwing (Erdoğan & Perez, 2015). It was noted that the row of caudally directed papillae of the caudal border of the laryngeal prominence demarcates it from the esophagus as in several avian species including Hume's tawny owl and common kestrel (Abumandour & El-Bakary, 2017) and house sparrow (Abumandour, 2018)

Conclusions

The study anatomically investigated the lingual and oropharyngeal structures of red-eyed dove that enable adaptations to granivorous feeding in the wild. It showed that the tongue and oropharyngeal features did not differ significantly from the closely related species in the order Columbiformes and some Galliformes. It demonstrated the presence of a short and narrow triangular-shaped tongue, cornified multilayered epithelium with high rate of desquamation of the epithelium of the tongue, prominent papillary crest, entoglossum, typical laryngeal mound, lingual and pharyngeal salivary tubuloalveolar lobules with acidic and neutral mucin contents. These features help the dove to adapt to grip, moisten, and swallow seeds. However, these structural features did not differ significantly from closely related species of Columbiformes and some Galliformes, but showed significant variations from those of some carnivorous birds and those feeding in water such as ducks, geese, and storks that carry out filter feeding, transport of food into the esophagus, and pecking behaviors.

Availability of Data and Materials

The data that supported the current findings are available from the corresponding author upon reasonable request.

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Author Contributions Statement

C.O.I. and F.E.M. conceived the study and participated in the experimental design. C.O.I. and U.M.B. performed the laboratory work, analysis of data, and photomicrography. C.O.I. wrote the initial draft of the manuscript. F.E.M. was involved in the critical review for important intellectual content and editing of the manuscript. All authors revised the final article's intellectual content and approved the final version for submission. All three authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work will be correctly investigated and resolved.

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Conflict of Interest

The authors declare that they have no competing interest.

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