ABSTRACT

There are sketches of data available on the penis of Megachiroptera. However, there is a dearth of information on the structures of the penis and its associated glands in the adult male *Epomops franqueti*. The penis is derived from mesoderm, which then differentiates into erectile tissues, connective tissues, smooth muscles, bones, and cartilage in a definite pattern to aid the penile function. The mammalian penis varies across different species in terms of penile shape, length, and appendages. Therefore, this work was aimed at investigating the anatomy of the African fruit bat’s penis and its associated glands. Ten adult male bats were captured and used for this study. The penises were dissected and processed for light microscopy. They were stained with hematoxylin and eosin (H&E) and Mason’s trichrome (MT). *Epomops franqueti* has an average penile length of 6.20 ± 1.10 mm and a width of 3.56 ± 0.20 mm. The penis of *Epomops* franqueti has a pair of corpora cavernosa that is more prominent than the single corpus spongiosum that surrounds the urethra. The prepuce of *Epomops franqueti* is retractable over the glans penis and it has preputial glands. The glans penis of the bat has no traces of baculum but it is furnished with accessory corpus cavernosum. Bulbourethral gland with simple columnar epithelium is located on the root of the penis, and this finding is novel in this species of bat. Gross and histological investigation reveals that *Epomops franqueti* has a vascular penis which is typical to mammals.

Keywords: Corpus cavernosa, Accessory Corpus cavernosum, Epomops franqueti, Penis, Prepuce
INTRODUCTION

Recently, some aspects of the reproductive tracts of the African fruit bat were studied to understand and provide basic information on the reproductive biology of *Epomops franqueti* (Ekeolu *et al.*, 2020, Ekeolu *et al.*, 2022). Therefore, investigating the bat penis and its associated glands will provide more veritable information to further understand *E. franqueti*’s reproductive biology.

Generally, there are reports on the penis of bats (Krutzsch, 1979, Welbergen, 2010, Herdina *et al.*, 2015) with sketches of data are available on Megachiroptera (Madkour, 1976, Nwoha *et al.*, 2000, Danmaigoro *et al.*, 2014), and there is dearth of information on the detailed structures of the penis and its associated glands in the adult male *E. franqueti*. The knowledge of the basic anatomy both at gross and histology level, of this African fruit bat penis, will complement the existing anecdotal knowledge as well as bridge the gap of information on the penile anatomy and its associated glands in *E. franqueti* and other species which will be a useful tool to the wildlife conservationist and laboratory scientists.

Therefore, there is a need for the investigation into the anatomy of the African fruit bat’s penis and its associated glands. Generally, the mammalian penis is a protruding structure that is used for delivery of sperm cells into the female reproductive organs during copulation (Hosken and Stockley, 2004). The penis is derived from mesoderm, and it develops in a species-specific pattern. This species variation of the mammalian penis aid their function (Mantione, 2005, Simmons and Jones, 2007, Weiss *et al.*, 2012) in terms of penile shape, length, and appendages (Simmons and Jones, 2007).

The penis is composed of erectile tissue: corpora cavernosum and corpus spongiosum and it is generally divided into root, body, and glans penis (Nwoha *et al.*, 2000). The corpus cavernosa is covered by tunica albuginea. In higher mammals, the tunica albuginea of the corpora cavernosa is two-layered: a complete inner circular layer and an incomplete outer longitudinal layer (Hsieh *et al.*, 2012). The mammalian penis is richly supplied with blood vessels. The deep dorsal vein in the mammalian penis is located between a pair of dorsal arteries (Hsieh *et al.*, 2012). The paired corpora cavernosa, the first erectile tissue is located on the dorsal aspect while the corpus spongiosum, the second erectile tissue, is on the ventral aspect of the penis (Dinotta *et al.*, 2013). Each of the paired corpora cavernosa penis, is covered by ischiocavernosum muscle. They form the central shaft of the penis (Krutzsch, 2009). The third erectile tissue, corpora cavernosa urethra (corpus spongiosum urethra) is covered by bulbospongiosus muscles that extend through the ventral length of the penis, lodging in the urethral groove between the junction of the rounded crura of the paired corpora cavernosa bodies. The corpus spongiosum terminates distally as the glans penis (Krutzsch, 2009).

The anatomy of the glans penis varies across bat species. The epithelium of the glans penis in bats is usually dermal, thick keratinized stratified squamous epithelium, with a basally directed spine as seen in Rousettus aegyptiacus, and the glans penis is short and narrows (Fard and Ghassemi, 2017). In Eidolon helvum, the presence of os penis has been reported (Nwoha *et al.*, 2000). The os penis modification of the glans penis helps to support the penis during ejaculation. It may also help to stimulate the female reproductive tract, preventing urethral closure during copulation (Hill and Harrison, 1987). The glans penis is covered by thick, hairy and has been suggested that the prepuce also contains erectile tissue known as accessory corpus cavernosum in some species of bat, while the urethra is covered by corpus spongiosum (Herdina *et al.*, 2015). The urethra opens through the glans penis as the external urethral orifice (Krutzsch, 1975). The structural variations in the penis of mammalian species including bats, are also
associated with the absence or presence of glands at the root, or body of the penis, or around the skin (prepuce) of the glans penis (Sohn et al., 2022). Some bat species have penile appendages like the os penis in fruit and insectivorous bats (Nwoha et al., 2000, Krutzsch and Crichton, 1987). The variations in the penises of bats are also seen in position and orientation. In the fruit bat, Rousettus aegyptiacus, the penis is elongated, cylindrical, and pinkish. It projects cranially (Fard and Ghassemi, 2017) and in Eidolon helvum it is also cylindrical and project cranially but pendulous (Nwoha et al., 2000).

The glands associated with the bat’s penis include the preputial, bulbourethral, and urethral glands of Littre. In some bats, the prepuce is furnished with mucoid glands. Bulbourethral glands are small and pear-shaped. They are present in almost all bat species reported so far (Krutzsch, 2009). They are located lateral to the root of the penis on both sides, encapsulated by fibromuscular tissue in neotropical bats. They are lobulated by connective tissue and skeletal muscle septae (Miotti et al., 2018). A pair of inguinal bulbourethral glands have been reported in Myotis nigricans (Negrin et al., 2013) unlike the extra-abdominal pair of bulbourethral glands reported in Artibeus lituratus. The multiple acini are lined by cuboidal epithelial cells with centrally or basally placed nuclei. These secretory cells are characterized by cytoplasm that has large membrane-bound secretory granules (Santos et al., 2018). This single duct of the gland passes beneath the bulbo-spongiosum muscle to the terminal bulb of the penile urethra distal to the prostate (Pal, 1983). Although the ducts are generally lined by cuboidal cells, in Mormopterus planiceps they are lined by sebaceous epithelial cells (Krutzsch and Crichton, 1987). The glands also are hypertrophied and secretory with granular, columnar epithelium, during the period when the bats are reproductively active (Pal, 1983).

The urethral gland of Littre is not universally present in bats but has been reported in Rhinolophids and Megadermatids (Krutzsch, 2009). Where present, this gland covers the membrane of the urethra as it is a highly developed, carrot-shaped gland. The tubules of the glands are highly convoluted and branching. It helps in the formation of vaginal plugs (Uchida and Mori, 1987, Krutzsch et al. 1992). Two types of cells line the epithelium of this gland: The urethral A cells which are columnar and much more granular with highly eosinophilic cytoplasm; the urethral B cells are pyramidal and basal in position with less granular and pale eosinophilic cytoplasm (Pal, 1983, Benard, 1986, Krutzsch et al., 1992).

The Littre gland is dispersed in the connective tissue of the urethra while the bulbourethral gland is located at the penile root (Santos et al., 2018). A Littre gland is small. It is a tubular structure that is lined by columnar, secretory epithelial cells. The nuclei of these cells are spherical which could be centrally or basally located within the cytoplasm (Pal, 1983).

MATERIALS AND METHODS
Ten adult male bats were captured using a mist net and used for this investigation following the method of Ekeolu et al. (2020). The body weights of the bats were taken using Microvar® weighing balance (Microvar, U.K). The body length and penile length and width were measured using a Draper® 115mm vernier caliper and metric tape. The animals were anesthetized using ketamine HCl (Biotechnica Pharma Global (BPG), China) at 25 mg/kg body weight intramuscularly on the medial side of the thigh muscle. The animals were sacrificed by cervical decapitation. The penis was dissected and immediately put in a petri dish of normal saline. The prepuce was retracted to expose the glans penis and then observed with the naked eye and hand lens. Also, the layers of the penis from the skin were dissected to reflect the inner surface of prepuce for observation. The penis was fixed in 4% buffered formalin. The
specimen was embedded in paraffin wax. It was processed for light microscopy by making transverse and longitudinal sections of 5µm thickness. It was stained with Hematoxylin and Eosin (H and E) and Masson Trichomes (MT) stains.

RESULTS

The penis of the *Epomops franqueti* appears to be rather stout with a cylindrical shaft, oriented cranially and covered by folds of skin, prepuce. The whole length of the penis (LP) was $6.20 \pm 1.10$mm, about three times the length of the glans penis (GP), $1.72 \pm 0.23$mm, and twice the size of the width of the penis (WP), $3.56 \pm 0.20$mm (Table 1). The prepuce has a median cleft proximally and bears sparse hair externally relative to the body hair. Gross observation revealed no visible preputial glands on the inside of the prepuce. The glans penis was larger than the body of the penis and completely hidden by the prepuce. The body of the penis arose from the brim of the fused the pubic bone on the ventral aspect of the pelvis (Fig. 1: A, B and C). The penis was composed of the body proximally and the glans penis distally. The penile body consists of a pair of corpora cavernosum and penile urethra. The pair of corpora cavernosa is separated by tunica albuginea septum. The cavernous tissue extends the whole length of the penis to reach the glans penis. The distal glans penis consists of corpora cavernosum and does not contain accessory erectile tissue. The glans penis is conically rounded in shape, bearing a centrally placed external urethral orifice, and it is devoid of surface papillae, appendages, or bones (Fig. 1: A, B and C, Fig. 2: A, B and C).

The ischiocavernous muscles in *E. franqueti* extend cranially in an oblique fashion from the ischium of the pelvis to insert into the fascia of the root of the penis. The bulbocavernosus muscle was not observed. The retractor penile muscle extends along the length of the corpus cavernosum (Fig. 2: A, B and C).

Light microscopy shows that the penis of *E. franqueti* is surrounded by a fibrous connective tissue, tunica albuginea, and composed of a pair corpora cavernosa and corpus spongiosus which lines the urethra. The corpus cavernosum extends into the glans penis as the accessory body. The preputial skin is lined by cornified stratified squamous epithelium which reflects upon itself to also line the inner surface of the prepuce. Part of the urethra is also lined by this epithelium. For the glands that are associated with the penis, the urethral gland of Litter around the urethra and ampullary glands in the *E. franqueti* were not revealed. Bulbourethral gland was observed at the root of the penis. The acini of the gland were numerous and conspicuous. The glands. The bulbourethral gland is composed of simple columnar non-ciliated epithelium with a round nucleus at the basal part of the cell. Each acinus is invested with connective tissue. The bulbourethral gland is surrounded by skeletal muscle. The corpus cavernosum tissue at the body and root of the penis is rich in blood vessels and numerous sinusoids (Fig. 3, A, B, C and D).

| Table 1. Mean and SEM of the weight of the animal and parameters of the penis of *E. franqueti* |
|---------------------------------|----------|----------|----------|
| WA (g)                      | LP (mm)  | GP (mm)  | WP (mm)  |
| 98.32 ± 1.29                | 6.20 ± 1.10 | 1.72 ± 0.23 | 3.56 ± 0.20 |

WA: Weight of the animal, LP: whole Length of the penis, GP: Whole length of glans penis, and width of the glans penis.
Figure 1. Photograph of the penis of AFB (E. franquetii), showing in (A) preputial covering of the penis (PC) and the preputial opening (PO). In (B), the pubic bone (PB), prepuce (PP), root of the penis (RP) with its fascia inserting on the pubic bone, glans penis (GP). In (C) the diagrammatic representation of the penis in (A)

Figure 2. Photomicrograph of the penis of AFB (E. franquetii), showing in (A) the right and left testes (RT&LT), fascia of the root of the penis (FS), bulbo spongiosus muscle (BS), ischiocavernosum muscle (IS), penis (PN), whole length of the penis (PL), width of the penis (PW). In (B) the right and left testes (RT&LT), the penis with tunica albuginea (TA) exposed, the retractor penile muscle (RP), septum (ST) of the tunica albuginea, and the urinary bladder (UB). In (C), exposure of the paired corpora cavernosa (CC) and corpus spongiosum erectile tissue (CS)
The average length of the penis of *E. franqueti* is 6.20 ± 1.10mm is less than the average length of Myotis with a size of 9.68 ± 0.75mm which is a microchiroptera indicating that the penis size may have no phylogenetic implication as *E. franqueti*’s penis size is approximately the same with that of Plecotus auritus with a penile length of 6.84 ± 0.79mm, even though they belong to different phylogenic order (Fasel et al., 2019). The retractor penile muscle present in the *E. franqueti* suggests that the muscle may help in the independent movement of the penis during copulation with less movement of the hip. The folds of skin, prepuce in the African fruit bat (AFB), *E. franqueti*, exposes the glans penis when retracted. The prepuce in the *E. franqueti* is rather glandular. The preputial glands help drain into the preputial space suggesting that it helps to keep the glans penis moist within the prepuce. The discharge substance from the preputial gland could also act as pheromones. The finding is like the report on the prepuce and glans penis in mice (Weiss et al., 2012). There were no traces of urethral gland of Litter in this bat species, however, light microscopy revealed bulbourethral gland at the proximal penis with numerous acinus which is lined by simple columnar epithelium.
and preputial glands at the distal penis. These findings correspond to the reports of Sohn et al. (2023) on the Greater horseshoe bat’s associated penile glands.

Penile appendages help to sustain an erection and aid successful mating (Nwoha et al., 2000). The glans penis of *E. franqueti* lacks appendages or fibrocartilages. The absence of the os penis in *E. franqueti*’s penis is unlike the findings in *Eidolon helvum* (Nwoha et al., 2000), in small rodents such as mice (Simmons and Firman, 2013) and large mammals such as dogs (Dixson, 2015). The glans penis of *E. franqueti* present accessory erectile tissue similar to the reports on accessory cavernosa tissue in Vespertilionid bats (Jubilato et al., 2019) and some Molossid bat species (Comelis et al., 2018) while in the other species of bat like the Carollia perspicillata the glans penis is furnished with striated musculature and adipose tissue (Jubilato et al., 2019). The accessory corpus cavernosum in the penis of *E. franqueti* may help in the provision of turgidity in the glans penis during copulation in a similar fashion the baculum, which its penis lacks, will provide support to the glans penis and prevents the urethral from collapsing during copulation as it has been reported in *Pipistrellis natthusii* (Herdina et al., 2015). The presence or absence of baculum penis among the Pteropodid may not necessarily define phylogeny as baculum has been reported in *Eidolon helvum*, a pteropodid while it is lacking in *E. franqueti* (Nwoha et al., 2000) and Molossid bat, a non-Pteropodid (Ryan, 1991).

The dome shape of the glans penis in this species of bat may be adapted to the adult female *E. franqueti*’s vagina for successful sperm deposition. The erectile tissue, corpus cavernosum, is paired and dorsal to the corpus spongiosum that covers the urethra. Also, the penis is surrounded by smooth and skeletal muscles, and rich in blood supply. The erectile tissue is also characterized by abundant connective tissue with abundant collagen fibers. This corresponds to the investigations carried out on Greater horseshoe bats (Sohn et al., 2021).

The urethral in *E. franqueti* penis extends from the proximal to the distal penis reaching the tip of the glans penis. This observation is typical in Chiropteran penis and other mammalian species like monkeys (Prakash and Ibrahim, 2018; Sohn et al., 2021). The paired corpora cavernosa is covered by tunica albuginea and separated by the tunica albuginea forming a septum between the two erectile tissues. Also, the corpora cavernosa is separated from the corpus spongiosum by connective tissue similar to the report of Comelis et al. (2018) on the penis in the Molossids.

**CONCLUSION**

The penis of *E. franqueti* is mainly composed of three erectile structures, which are: A pair of corpora cavernosa and a single corpus spongiosum, with an accessory corpus cavernosum in the glans penis. The investigation of the penis of *E. franqueti* shows that the vascular and hydrostatic morphology of the penis of *E. franqueti* is mostly similar to that of other Chiroptera and mammalian species.

**REFERENCES**


