Original Research Article

TITLE: Anatomical assessment of the eye of the African grasscutter (*Thryonomysswinderianus*)

SUMMARY

Purpose: To determine the macroscopic and microscopic ocular morphological characteristics of the African grass-cutter

Materials and Methods: Ten male grass-cutters of mean age 4.05 ± 1.44 months and mean weight 1.04 ± 0.56 kg were used for this study. Gross morphologic and light microscopic techniques were employed in the study of the eyes.

Results: Grossly, the eye exhibited typical characteristics of the mammalian eye with a mean eye weight and mean corneal diameter of 0.47 ± 0.14 g and 0.73 ± 0.07 cm, respectively. The horizontal corneal diameter was significantly greater ($p < 0.05$) than the vertical corneal diameter, and the ratio of mean corneal diameter to mean eye diameter (MCD:MED) was 0.80. The sclera and corneal stroma were dense fibrous connective tissues and had thicknesses of 105.3 ± 25.8 µm and 201.4 ± 91.3µm, respectively, while the corneal epithelium was stratified squamous epithelium and measured 50.1 ± 15.1µm. The choroid, ciliary stroma, and iridal stroma were pigmented connective tissues, while the retina was a multi-layered neuro-epithelial tissue with scanty ganglion cells and a retinal pigment epithelium that was pigmented throughout its length.

Conclusion: The high MCD:MED and scanty retinal ganglion cells observed are associated with nocturnal visual capability. However, the complete pigmentation of the retinal pigment epitheliumsuggest the absence of tapetumlucidumin this species. This could considerably lower its nocturnal visual capability and indicate a low reliance on vision for environmental perception. The biometrical measurements obtained have made data available for use in future ocular studies of the rodent.

Key words: Ocular morphology,hystricomorpha,rodent, *Thryonomysswinderianus*

1. Introduction

The African grasscutter is a nocturnal rodent of the family thryonomidae and suborder hystricomorpha(Igbokwe, 2010). It is usually found in dark environments, such as within lush vegetation and in burrows at daytime. It has gained attention in West Africa as an alternative source of meat and income(Akinola*et al*., 2015) The increased demand for the meat of this wild rodent aided by its ability to reproduce in captivity have led to the increase in grasscutter farming all over the West African subregion. The sustained growth of this industry may however be impeded by lack of basic knowledge of the biology of the grasscutter. Scientific data on the morphology of the eye of the grasscutter would be useful in its behavioural and medical management, especially in the recognition of ocular pathology.

Though numerous studies have been conducted on the grass-cutter (Ajayi*et al*., 2012; Obadiah*et al*., 2015; Olukole & Obayemi, 2010), studies related to the eye of the rodent are yet very scanty. Therefore, this study sought to describe the ocular characteristics of the African grass-cutter using gross morphologic and light microscopic techniques, and to make available for future reference, its ocular biometric features.

2. Materials and methods

2.1. Experimental animals

All procedures that involved animals were conducted according to stipulated guidelines for the protection of animal welfare in the University of Nigeria, Nsukka.

Ten male African grass-cutters of mean age 4.05 ± 1.44 months and mean weight 1.04 ± 0.56 kg were used for this study. They were obtained from Demacco Farm, Nike, Enugu East Local Government Area, Enugu State, Nigeria, where they were raised in a scarcely illuminated environment.

2.2. Gross anatomy

Following sedation of the rodents using intramuscular injection of xylazine hydrochloride (7 mg/kg), horizontal and vertical corneal diameters were obtained from each eye using Vernier caliper. Euthanasia was subsequently achieved using intramuscular injection of ketamine hydrochloride (120 mg/kg). Eyes were bilaterally enucleated(Hall, 2008) and the eye weight as well as the horizontal, vertical, and axial eye diameters were obtained. The physical appearance and topography of the eyes were studied.

2.3. Light microscopy

Whole eyes were fixed in Davidson's fixative(Agrawal *et al*., 2007) for 18 hours and subsequently post fixed in 10% neutral buffered formalin. The samples were routinely processed for light microscopy and stained with haematoxylin and eosin (H&E) and Masson's trichrome stains. Photomicrographs were obtained using Moticam Images Plus 2.0 digital camera (Motic China Group Ltd., China) and the thicknesses of the sclera, cornea and retina were measured from one eye of each animal. Due to the varying thicknesses across the length of each parameter, five random locations were used for each measurement and the means and standard deviations were obtained.

2.4. Data analysis

Data were analyzed out using SPSS Statistics 17.0 software. Data were presented as mean ± SD. Mean corneal diameter was calculated as the mean of all horizontal and vertical corneal diameters while mean eye diameter was calculated as the mean of all horizontal and vertical eye diameters. The Wilcoxon signed ranks test was used to determine any significant differences between the vertical and horizontal corneal diameters, vertical and horizontal eye diameters, vertical and axial eye diameters, and axial and horizontal eye diameters. Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Gross anatomy

Grossly, the eye exhibited typical characteristics of the mammalian eye (Figure 1). Each eye was located laterally in the orbital cavities of the skull with an anterior transparent cornea and a posterior slightly translucent whitish sclera. The sclera was divided into anterior and posterior parts by the whitish translucent conjunctiva, which adhered tightly to the anterior part. Extraocular skeletal muscles were attached to the posterior part. A dark golden-brown iris surrounding a vertically-oriented oval pupil was visible through the cornea. The optic nerve extended from the posterior part of the eye as a white thread-like structure surrounded by extraocular muscles (Figure 2).

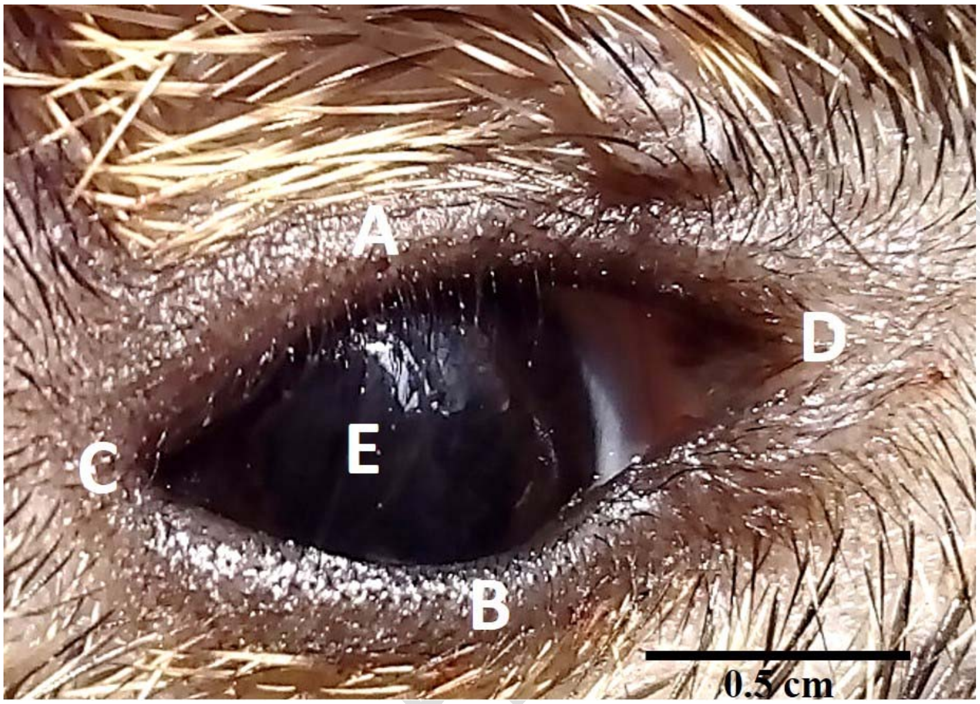

Figure 1: Gross photograph of the right eye of *Thryonomysswinderianus* showing typical features of the mammalian eye. Upper eyelid (A), lower eyelid (B), lateral canthus (C), medial canthus (D), eyeball (E).

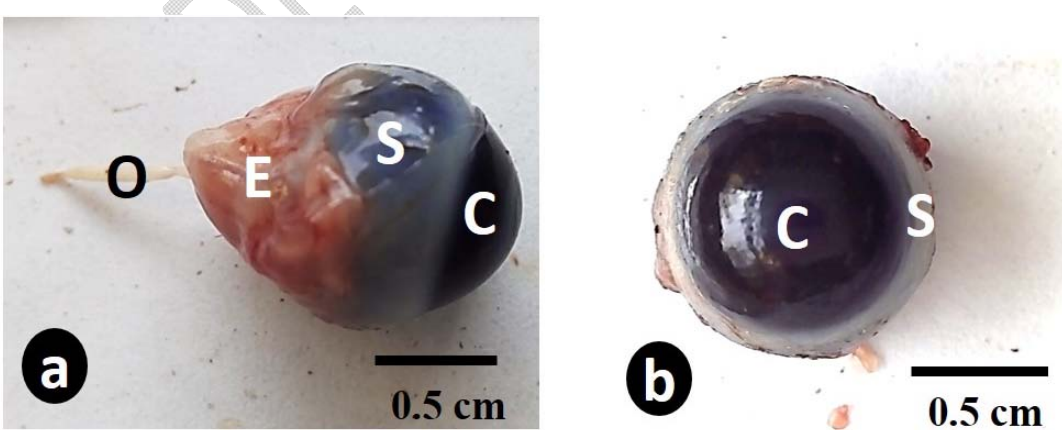

Figure 2: Gross photograph of the enucleated eye of *Thryonomysswinderianus*. Figure 2a shows the equatorial view while figure 2b shows the anterior view. Cornea (C), sclera (S), extraocular muscles (E), optic nerve (O).

The mean eye weight was 0.47 ± 0.14 g, while the mean corneal diameter was 0.73 ± 0.07 cm. The horizontal corneal diameter (0.74 ± 0.08 cm) was significantly greater ($p < 0.05$) than the vertical corneal diameter (0.71 ± 0.05 cm). The horizontal eye diameter (0.93 ± 0.07 cm) and axial eye diameter (0.92 ± 0.08 cm) were not significantly different ($p > 0.05$) from each other, but were significantly greater ($p < 0.05$) than the vertical eye diameter (0.90 ± 0.09 cm). The ratio of mean corneal diameter to mean eye diameter was 0.80, while the ratio of mean corneal diameter to mean axial eye diameter was 0.79.

3.2. Histology

3.2.1. Fibrous tunic

The sclera was a dense fibrous connective tissue. It contained numerous fibrocytes and collagen fibers. Pigment cells were scanty and located posteriorly. The cornea appeared as a regular dense fibrous connective tissue (corneal stroma) lined internally by simple cuboidal epithelium (corneal endothelium) and externally by non-keratinized stratified squamous epithelium (corneal epithelium) (Figure 3). The stratified epithelium was 5-12 cell layers thick with round basal cells, oval middle cells and flat apical cells. Unstained perinuclear areas were common among the basal and middle cells. A deeply acidophilic thin area, known as the Descemet's membrane, was observed between the stroma and endothelium (Figure3b). The stroma was filled with closely packed collagen fibers (Figure 3c).The corneal endothelium, stroma, and epithelium made up about 3%, 78%, and 19% respectively of the entire corneal thickness (Table 1).

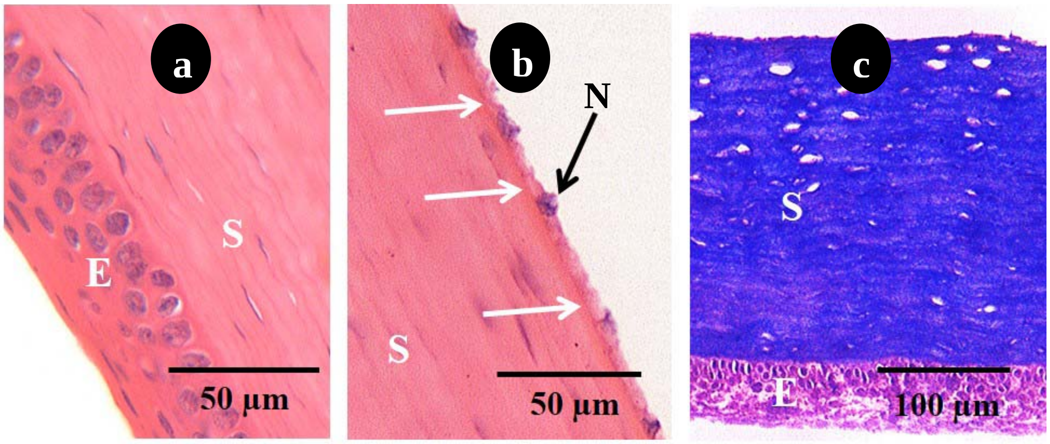

Figure 3. Photomicrograph of the cornea of *Thyronomys swinderianus*. Figures 3a and 3b show the corneal epithelium (E), corneal stroma (S), cells of the corneal endothelium (N) and the deeply acidophilic, thin Descemet's membrane (white arrows). Haematoxylin and eosin stain Figure 3c shows the corneal epithelium (E) and corneal stroma (S) filled with collagen. Masson's trichrome stain

Table 1: Corneal, scleral, and retinal thicknesses of the African grasscutter

	Mean ± SD (µm)	**Range (µm)**	**Percentage**
Cornea	257.5 ± 105.9	123.9 - 399.3	-
Corneal endothelium	7.3 ± 1.8	4.9 - 11.2	3%
Corneal stroma	201.4 ± 91.3	86.6 - 329.0	78%
Corneal epithelium	50.1 ± 15.1	25.3 - 74.4	19%
Sclera	105.3 ± 25.8	61.5 - 151.8	-
Retina	140.9 ± 19.4	110.9 - 226.4	-
Retinal piment epithelium	11.1 ± 2.5	7.0 - 16.0	8%
Photoreceptor layer	26.5 ± 7.6	13.3 - 39.7	19%
Outer nuclear layer	31.4 ± 8.0	19.4 - 45.1	22%
Outer plexiform layer	8.1 ± 2.9	4.0 - 12.6	6%
Inner nuclear layer	17.7 ± 3.6	13.0 - 26.7	13%
Inner plexiform layer	24.0 ± 5.2	17.2 - 34.8	17%
Layer of ganglion cells and axons	23.5 ± 3.5	18.1 - 30.1	17%

3.2.2. Uvea

The uvea comprised the iris, ciliary body, and choroid. The choroid and ciliary body were separated at the oraserrata (Figure 4). The ciliary body was not well developed. Its stroma, which was continuous with the choroid, was a vascularized pigmented connective tissue layer. Smooth muscle fibers were not observed in the ciliary stroma. The ciliary epithelia consisted of an outer pigmented epithelium and an inner non-pigmented epithelium. The non-pigmented epithelium was simple cuboidal towards the apex of the ciliary processes, but varied from simple columnar to stratified cuboidal towards the base. However, the non-pigmented epithelium gradually accumulated dark brown melanin pigments towards the iris. The connective tissue of the ciliary stroma at the base of the ciliary processes appeared as a meshwork of fibers and cells, the trabecular meshwork (Figure 5). It lacked muscle tissue. Anterior to the ciliary processes was the iris, a long process that extended from the base of the ciliary processes to the space anterior to the lens (Figure 4). It was made up of pigmented connective tissue lined posteriorly by pigmented epithelium that was continuous with the ciliary epithelia.

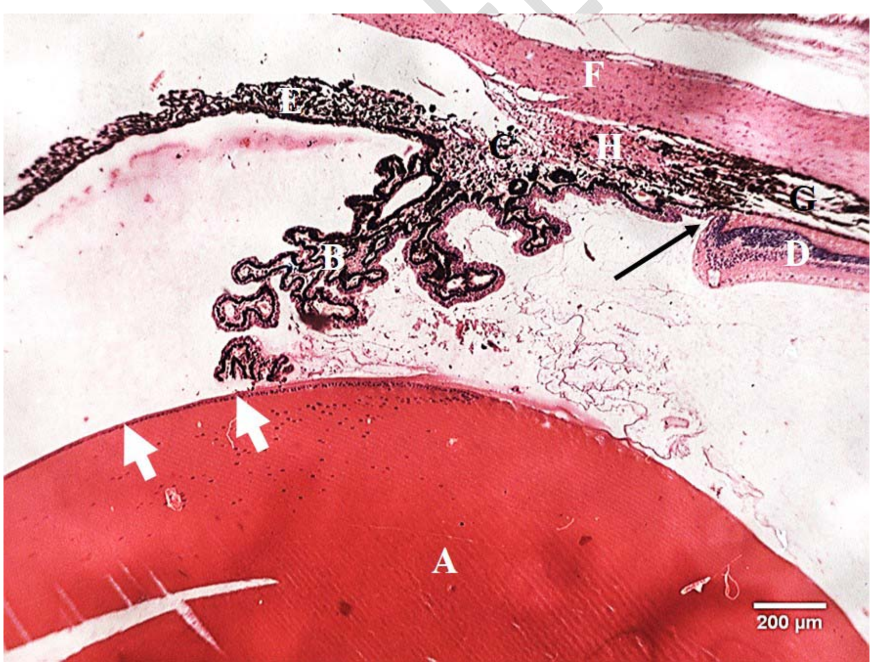

Figure 4. Photomicrograph of the eye of *Thyronomys swinderianus*. Lens (A), ciliary processes (B), trabecular meshwork (C), retina (D), iris (E), corneoscleral junction (F), choroid (G), ciliary stroma (H), lens epithelium (white arrows), ora serrata (black arrow). Haematoxylin and eosin stain

Figure 5. Photomicrograph of the ciliary body of *Thyronomys swinderianus*. Corneoscleral junction (J), ciliary processes (P), ciliary stroma filled with trabecular meshwork (M) and devoid of muscle tissue. Haematoxylin and eosin stain

3.2.3. Retina

The internal surface of the choroid was lined by multi-layered neuro-epithelial tissue known as the retina (Figure6). At the base of the retina was a layer of low simple cuboidal epithelium, the retinal pigment epithelium. The apical three-quarters of the cytoplasm of the epithelial cells were filled with dark brown melanin pigments. Beneath this epithelium was an acidophilic layer, the photoreceptor layer. The outer nuclear layer comprised numerous round heterochromatic nuclei separated by unstained inter-nuclear spaces (Figure 6).The outer nuclear and photoreceptor layers were the thickest layers of the retina, occupying about 22% and 19% respectively of the retinal thickness (Table 1). Two acidophilic layers (outer and inner plexiform layers) were subjacent to the outer nuclear layer. The outer and inner plexiform layers were separated by an inner nuclear layer of mostly round euchromatic nuclei and few elongated euchromatic nuclei. Nuclei of the outer nuclear layer appeared to make contact with the thin outer plexiform layer through numerous acidophilic fiber strands. Unstained inter-nuclear spaces were also observed in the inner nuclear layer. The apical layer of the retina contained sparse euchromatic nuclei of ganglion cells dispersed among numerous acidophilic axons. These layers of the retina thinned down abruptly and merged with the ciliary epithelia at the oraserrata.

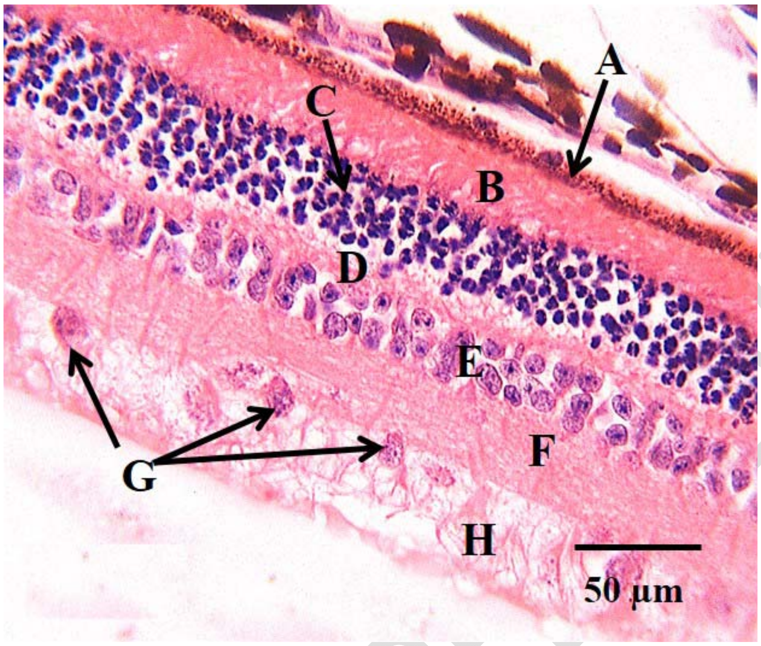

Figure 6. Photomicrograph of the retina of *T. swinderianus*. Retinal pigment epithelium (A), photoreceptor layer (B), outer nuclear layer (C), outer plexiform layer (D), inner nuclear layer (E), inner plexiform layer (F), ganglion cell nuclei (G), axons (H). Haematoxylin and eosin stain

3.2.4. Lens

The lens was a large, deeply acidophilic, circular structure (Figure 4). It was located in the anterior part of the eye between the ciliary processes. A simple cuboidal epithelium, the lens epithelium, lined the anterior surface of the lens while the posterior surface had no epithelial lining. Numerous elongated lens fibers filled the lens.

4. Discussion

Morphology of the cornea of the African grasscutter showed similar features as the cornea of domestic mammals(Hamor & Ehrhart, 2006; Nautscher*et al.*, 2016). The closely packed abundant collagen fibers observed in the cornea and sclera of the African grasscutter may serve to provide the tensile strength needed to maintain ocular structural integrity irrespective of fluctuating intraocular pressure. Such amount and arrangement of fibers would prevent rupture of the eyeball in the event of anomalies associated with production and drainage of aqueous humour(Davis*et al*.,2015). The stratified squamous epithelial layer of the corneal epithelium together with the conjunctiva may serve as the anterior protective barrier for the eyeball, since both structures are directly exposed to the external environment. The unstained perinuclear areas observed in the corneal epithelial cells have been demonstrated in the dog, cat, goat, cow, and horse(Hamor & Ehrhart, 2006; Nautscher *et al*., 2016), but were absent in human cornea(Mescher, 2010; Muller*et al*., 1995, 1996). These may have been ignored or possibly dismissed as artifacts by previous authors. These unstained areas may not be artifacts, but may require further investigation.

The Bowman's membrane of the cornea has been demonstrated in primates and avians(Hamor & Ehrhart, 2006; Mescher, 2010; Muller *et al*., 1995), but was not observed in the grasscutter. The presence of this membrane in domestic mammals is questionable(Nautscher *et al*., 2016) and its function is not clearly understood. The ratio of mean corneal diameter to mean eye diameter and that of mean corneal diameter to axial eye diameter were relatively large and comparable to those of nocturnal and cathemeral mammals(Hall*et al*., 2012; Kirk, 2004). Such large ratios may increase visual sensitivity(Kirk, 2004) and permit more light rays into the eye at maximum pupillary dilation, unlike in diurnal mammals with smaller ratios(Hall *et al*., 2012). The significantly larger horizontal corneal diameter reported in this study and in most mammals(Augusteyn *et al*., 2012; Hamor & Ehrhart, 2006; Maggs, 2008; Plummer*et al*., 2003) may be interpreted as an adaptation to minimize energy expended in keeping the eyelids open. We postulate that such an adaptation may allow for entrance of considerable amount of light rays into the eye with minimal exertion of energy by the elevator and depressor muscles of the upper and lower eyelids respectively. The thickness of the corneal epithelium in the grasscutter is small when compared to those of other domestic animals(Nautscher *et al*., 2016). This may indicate existence of a positive correlation between corneal epithelial thickness and body weight across species.

The ciliary body described in this study is similar to that described in rabbits and rodents(Davis, 1929; Woolf, 1956). It was characterized by poorly developed ciliary body and therefore, possible absence of lenticular accommodation. Accommodation nonetheless appears irrelevant to nocturnal animalsin which visual sensitivity takes pre-eminence over visual acuity. Ciliary muscle fibers have however been reported in the mouse(Treuting*et al*., 2012). The presence of melanin pigments in the non-pigmented epithelium of the ciliary body at the para-iridal area suggests that this area may be a transition zone between the ciliary body and the iris. Thus, the term, 'non-pigmented epithelium' appears unsuitable for this area.

The sum of the percentages of the various retinal components was about 102% instead of 100% due to errors in the subjective determination of the measurements. This was because the entire retinal thickness stated was not a direct numerical computation of the thicknesses of its components but was rather measured alongside its components.The retinal pigment epithelium in the grasscutter exhibited melanin pigments throughout its length. In mammals with tapetumlucidum, the retinal pigment epithelium usually lacks melanin pigments over the central area of the tapetum(Ollivier *et al*., 2004). The presence of pigments throughout the tapetum therefore suggests the absence of tapetum lucidum in the grasscutter. Tapetumlucidum has been reported to be absent in most rodents except the spotted cavy (*Cuniculus paca*) and the springhaas (*Pedetescapensis*)(Fernandez & Dubielzig, 2013).It is a reflective structure of the eye that improves night vision in nocturnal, cathemeral and crepuscular animals.

The sparse ganglion cells in the retina of the grasscutter indicate poor visual acuity in this species. This feature, known as retinal pooling(Hall, 2008), has been associated with nocturnal animals.

In photopic animals such as humans, there is a higher density of ganglion cells, and consequently, high visual acuity(Vajzovic *et al*., 2012).

5. Conclusion

In conclusion, the visual system of the African grasscutter is adapted for nocturnal vision as suggested by the high ratio of mean corneal diameter to mean eye diameter and scanty retinal ganglion cells. This nocturnal visual capability may however be considerably lowered by the absence of tapetumlucidum as suggested in this species.The biometrical measurements obtained has made data available for use in future ocular studies of the rodent.

Disclaimer regarding Consent and Ethical Approval:

As per university standard guideline, participant consent and ethical approval have been collected and preserved by the authors.

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