

Observed sexual dimorphism in the epidermal gland structure and arrangement for the Fossorial Skink *Scelotes bidigittatus* FitzSimons, 1930

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Scelotes bidigittatus is a small burrowing scincid with an obligate fossorial lifestyle (Maritz and Alexander, 2008). It lives in sandy substrate and leaf litter in savannah, forest, and grassland habitats and ranges from the South African Lowveld through eSwatini to southern Mozambique and northern KwaZulu-Natal province, South Africa (Broadley, 1994; Branch, 1998; Jordaan, 2021). During August 2018, seven *S. bidigittatus* fatalities were collected during a post-fire mortality survey, following a late winter management fire on the Munywana Conservancy in KwaZulu-Natal province. Six of these specimens were prepared for histopathological examination to investigate fire-associated mechanisms of mortality (Jordaan and Steyl, 2023). During histopathological examination, variations in epidermal glands were noted between specimens suggesting possible sexually dimorphic differences in their structure, size, and spatial arrangement.

Methods

All collected specimens were mature (snout-vent length (SVL): 70–75 mm) following the adult size parameters in Branch (1998) (SVL: 60–80 mm) and had likely succumbed to the effects of noxious gasses originating from the fire (Jordaan and Steyl, 2023). Specimens were sectioned transversely into 5 mm thick samples for the

purpose of tissue fixation in 10% buffered formalin followed by routine histological processing yielding haematoxylin and eosin-stained sections for histological examination (Bancroft and Gamble, 2002). Of the six prepared specimens, four were in adequate condition to allow meaningful histological examination of the integument and other soft tissues. Since all visceral tissues were present, accurate sexing of specimens was achieved via the direct observation of either testes or ovaries. Direct correlations could thus be drawn between dermal structures and the sex of each specimen.

Results

Of the four specimens that were suitable for histological examination, two were male and two were female. Upon detailed histological examination of the dermal tissues using an Olympus BX40 light microscope at 100 and then 400 times magnification, specialised sub-scalar epithelial cells, forming gland-like epithelial clusters in the inter-scalar hinge regions, which varied in their morphometric and staining characteristics, were noted. These gland-like structures were circumferentially distributed throughout the integument, progressively decreasing in size and prevalence from anterior to posterior. A noticeable difference in composition and size of these gland-like structures was detected between sexes. Females exhibited less developed sub-scalar glands than did males and were composed of only one cell type (Fig. 1). Glandular structures of males were larger in volume and more numerous (Fig. 2), consisting of at least two different cell types (Fig. 3). No secretions were obtained or visualised from these glandular materials.

Discussion

Canei and Nonclercq (2020) described the distribution and structure of epidermal glands in two fossorial psammophilous scincids, *Scincus scincus* (Linnaeus,

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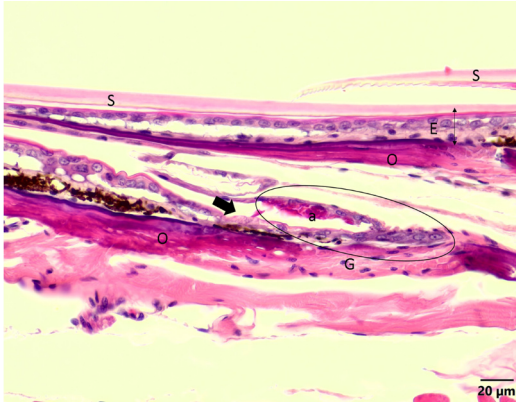


Figure 1. Skin of a female *Scelotes bidigittatus*. G (oval circle): Rudimentary glandular structure comprising monotypic glandular epithelial cells (a) located in the sub-scalar hinge region (solid arrow) communicating to the outer epidermal keratin layer (S) of each scale. Underlying the epidermis (E) are osteoderms (O). (Photomicrograph: haematoxylin & eosin staining; 400x magnification). Photo by J.C.A. Steyl.

1758) and *Eumeces schneiderii* (Daudin, 1802), which follow a very similar pattern to what we observed in *S. bidigittatus*. As the tissues from that study were derived primarily from male specimens, sexual variation was

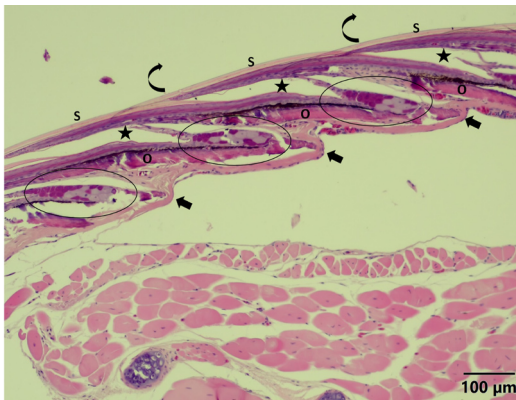


Figure 2. Skin of a male *Scelotes bidigittatus*. Well-developed complex glandular epithelial cells exhibiting varying staining characteristics (oval circles). The glandular structures are located in the sub-scalar hinge region (stars) communicating to the outer epidermal keratin layer (S) of each scale. Osteoderms (O) supporting the overlying epidermis (scale) originate from dermal connective tissue immediately distal to the glandular tissue (arrows). (Photomicrograph: haematoxylin & eosin staining; 100x magnification). Photo by J.C.A. Steyl.

not assessed, and these authors speculated that the structures and the secretions they produce are linked to the movement of these species through loose sands (Canei and Nonclercq, 2020). An additional difference with the finding in this study is that the glandular structures described by Canei and Nonclercq (2020) are classified as holocrine glands, whereas the classification of the structures in the present study is more complicated since secretory material could not be visualised. Although the absence of typical holocrine secretion in the current study could be an artefact of processing, the cellular composition differences between the gland-like structures for the different sexes suggest a more complex glandular classification possibly involving endocrine-, merocrine- and/or apocrine functions. The holocrine properties of glands in the former study are often associated, among others, with a lubricating function (Allam et al., 2016; Recio et al., 2023). The absence of typical histomorphological holocrine structures and the sex-associated cellular differences in the sub-scalar gland-like tissue of *S. bidigittatus* suggests an alternative, or additional, function of these structures - possibly related to scent or pheromonal production. Pheromone production and associated chemical secretions by various skin glands is well known for many reptiles including many Scincidae species (Weldon et al., 2008), with sexual dimorphism reported for glandular tissues in several groups of lizards (Rutland et al., 2019).

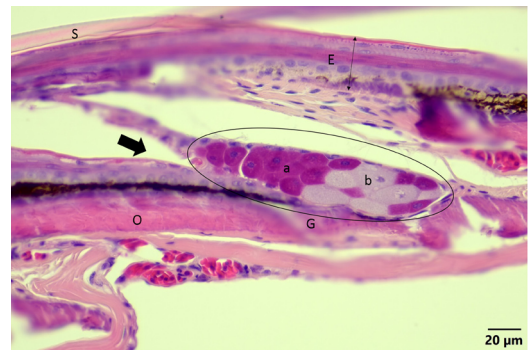


Figure 3. Skin of a male *Scelotes bidigittatus*. G (oval circle): Well-developed complex glandular structure comprising epithelial cells exhibiting varying staining characteristics of amphiphilia (a) and basophilia (b). The glandular structure is located in the sub-scalar hinge region (solid arrow) communicating to the outer epidermal keratin layer (S) of each scale. Underlying the epidermis (E) are osteoderms (O). (Photomicrograph: haematoxylin & eosin staining; 400x magnification). Photo by J.C.A. Steyl.

The variation in scent or pheromone production between the sexes of *S. bidigittatus* potentially has biological and ecological implications for the species and possibly other members of the genus. As sand or soil is in constant contact with the skin of fossorial species when moving through substrate (Allam et al., 2016), chemical tracks may be produced by individuals, most likely males in this case. If true, this may allude to the potential of male *S. bidigittatus* either advertising their presence or allowing females to seek out males for mating. Since both sexes have some similarity in the sub-scalar glandular structures, the potential exists that some alternative functionality of such secretions is probable. As suggested by Canei and Nonclercq (2020), these secretions may also affect physicochemical properties of the outer scales allowing for more efficient movement through substrate. The extent and potential function(s) of these structures in other *Scelotes* or fossorial skinks should be investigated further. Given that most of the threatened or Data Deficient skinks in South Africa are cryptic fossorial species (Tolley et al., 2019), the presence of scent glands may make the use of detection dogs potentially viable for their surveying or monitoring as has been proven for other subterranean reptiles elsewhere in the world (Nielsen et al., 2016).

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