

Preliminary studies on the morphology of the scent glands of soil-dwelling harvestmen (Arachnida: Opiliones)

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Abstract. Large prosomal scent glands are characteristic of all Opiliones. These glands are most conspicuously developed in the suborders Laniatores and Cyphophthalmi, but less so in the third classical opilionid suborder, the Palpatores. In the present study, the glands of several soil-dwelling opilionids were investigated by scanning electron microscopy and semithin-histological sectioning. Several different glandular types were detected: the *Trogulus*-type, as found in *Trogulus tricarinatus* (Linnaeus, 1767), is characterized by an external secretion-atrium covering the ozopores and the presence of solid balls of secretion in the scent gland reservoirs. The lumina of scent glands in *Anelasmacephalus hadzii* Martens, 1978 are filled with cobweb-like projections of the glandular epithelium. These glands also open into a secretion atrium. The scent glands of *Paranemastoma quadripunctatum* (Perty, 1833) exhibit some characteristic features of defensive glands, e.g. large reservoirs the intima of which are extensively folded. Ozopores, however, still lead into a laterally-opening secretion atrium. *Amilenus aurantiacus* (Simon, 1881), the only eupnoid species in this study, has scent glands with large secretory cells but comparably small glandular reservoirs. Unlike the hidden ozopores of all three soil-dwelling opilionids of the suborder Dyspnoi investigated in this study, the slit-shaped scent gland openings of *Amilenus aurantiacus* are exposed on a cuticular protrusion dorsal to legs I. The presence of distinct types of scent glands, especially in soil- (and cave-) dwelling Palpatores, may be indicative of multiple evolutionary traits that possibly resulted in their functioning as both scent and defence glands.

Key words. Soil zoology, morphology, histology, anatomy, Arachnida, Palpatores, Dyspnoi, Opiliones, chemical defense, scent glands.

INTRODUCTION

The presence of large prosomal exocrine glands, also called scent or repugnatorial glands, is an important synapomorphic character of all Opiliones. In general, these glands are thought to be for defence (Martens 1978) but may have other functions, e.g. production of pheromones (Holmberg 1986). Even though the scent glands are conspicuous and strikingly developed in the Laniatores and Cyphophthalmi, they are rather inconspicuous in many palpatorid harvestmen. Especially, in soil-dwelling opilionids, which are mainly dyspnoic Palpatores, the scent glands are hidden and inconspicuous, and it is difficult to induce them to produce secretion. In fact, in certain taxa such as in Trogulidae, the release of secretion has never been observed, not even following violent mechanical irritation (Pabst 1953, Schaidler & Raspotnig 2009). These observations not only indicate biological roles other than the generally assumed chemical defence but also a scent gland anatomy

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that clearly differ from the so far described allomonal types in other Opiliones (e.g. Gutjahr et al. 2006). In *Trogulus tricarinatus*, e.g. Schaidler & Raspotnig (2009) report solid boli of secretion in scent gland reservoirs, which are unlikely to be easily discharged. A comparable situation is reported for subterranean Ischyropsalididae (Juberthie et al. 1991). In these cases, it is suggested the secretion is released in gaseous form after sublimation from solid crystals (e.g. Gnaspini & Hara 2007). Based on these preliminary and speculative results and the general reluctance to discharge a secretion, it seems likely that the scent glands in many soil-dwelling opilionid taxa will have a completely different morphology.

However, as morphological data on opilionid scent glands are scarce the scent glands of several soil-dwelling palpatorid taxa (mainly representatives of Dyspnoi) were studied, using scanning electron microscopy and semithin-histological sectioning, in order to elucidate the morphological basis of the phenomena described above.

MATERIAL AND METHODS

Specimens of *Trogulus tricarinatus* Linnaeus, 1767, *Anelasmacephalus hadzii* Martens, 1978 (both Dyspnoi, Troguloidea, Trogulidae), *Paranemastoma quadripunctatum* Perty, 1833 (Dyspnoi, Troguloidea, Nemastomatidae) and *Amilenus aurantiacus* Simon, 1881 (Eupnoi, Sclerosomatidae) were extracted from soil samples and collected in caves at different locations in Carinthia and Styria (Austria), with the aid of a Berlese apparatus or by hand, respectively. Specimens were fixed in Bouin for 24 hours, washed, dehydrated and embedded in LR-white soft grade (Gröpl, Tulln, Austria). Embedded specimens were sectioned using glass knives and a rotary microtome (Leica Jung 2065, Leica, Vienna, Austria), which resulted in serial sections 2.5 µm thick. Sections were stained with toluidin blue (Lactan, Graz, Austria). Scent glands were reconstructed using the serial sections according to Honomichl et al. (1982) and 3d-reconstruction software (Amira 4.1), respectively.

For scanning electron microscopy (SEM), specimens were fixed in Bouin for 24h, washed in 70% ethanol, dehydrated, air-dried and mounted on aluminium stubs prior to sputtercoating (AGAR sputtercoater, Gröpl, Tulln, Austria). Micrographs (SEM) were taken with a Philips XL30 ESEM (Philips/FEI, Vienna, Austria) at high vacuum mode and 20kV accelerating voltage.

RESULTS

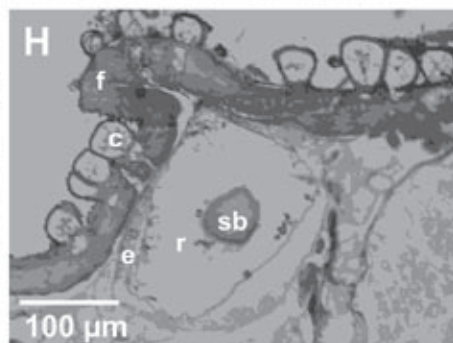
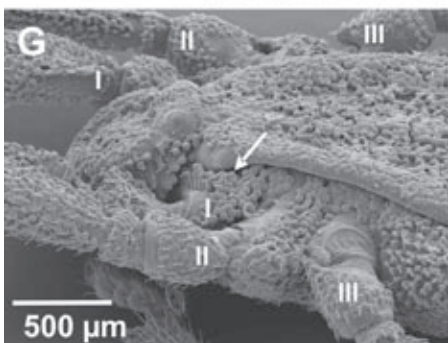
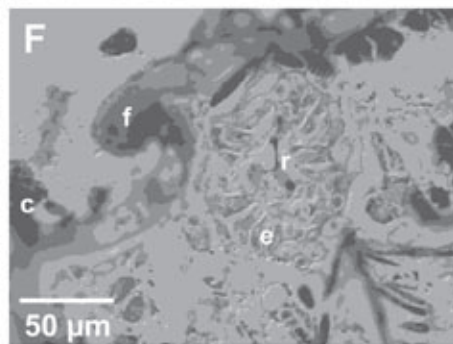
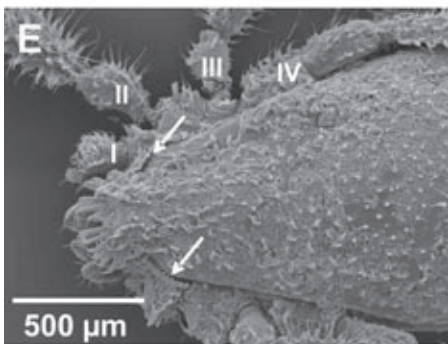
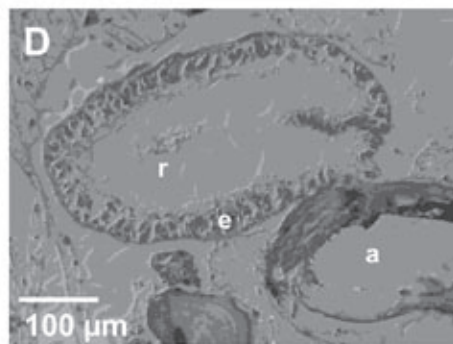
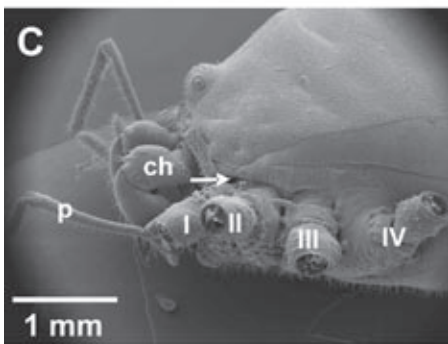
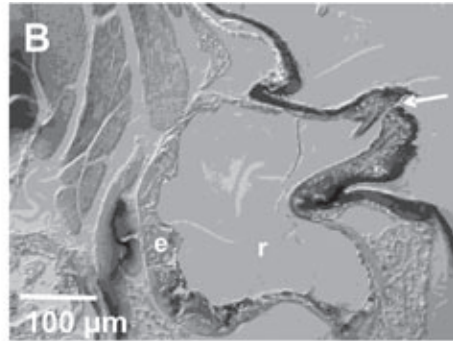
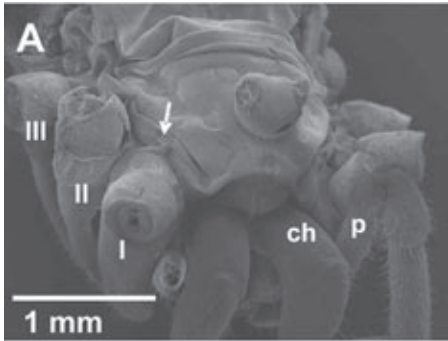
In all species investigated, the scent glands were well developed and consist of a pair of large sac-like glands on either side of the prosoma, extending backwards from the first pair of legs.

In *Trogulus tricarinatus* scent glands are s-shaped and in length about 10% of the body length. Ozopores are situated under a dorso-lateral integumental fold. This fold is the dorsal delimitation of an “atrium” that completely covers the ozopores. The remaining structures of this atrium are the dorsal parts of coxa I (ventral limitation) and a wall of cuticular papillae (lateral limitation). A narrow slit-shaped opening between the wall of cuticular papillae and the integumental fold leads to the exterior and thus to the surface of the body (Fig. 1G). Histologically, glandular epithelial cells are flat and elongated in the anterior part of the gland (= non-secretory area) and voluminous and full of granules in the posterior part (= secretory area). Spherical structures, obviously balls of solid secretion, are present in the lumina (Fig. 1H).

Similarly, the scent glands of *Anelasmacephalus hadzii* extend backwards for about 10% of body length. Scent gland openings are also located beneath a dorso-lateral fold of the integument and are very small and hardly noticeable using light microscopy. Openings lead again into an

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Fig. 1. Scanning electron microscopic (SEM) micrographs of the prosoma and cross sections through the scent glands of four species of Opiliones: A, B – *Amilenus aurantiacus*; C, D – *Paranemastoma quadripunctatum*; E, F – *Anelasmacephalus hadzii*; G, H – *Trogulus tricarinatus*. Arrows point to ozopore (A, B) or secondary openings (C, E, G). Abbreviations: I–IV – first to fourth pairs of legs, a – secretion atrium, c – cuticular papillae, ch – chelicerae, e – scent gland epithelium, f – dorsal integumental fold, p – pedipalpus, r – scent gland reservoir, sb – bolus of secretion.



external atrium made of parts of coxa I, the integumental fold and a wall of cuticular papillae (Fig. 1E). The gap between cuticular papillae and dorsal integument is larger than in *Trogulus* (in *T. tricarinatus* about 10 μm , here about 40 μm). Glandular epithelial cells are generally large and protrude into the lumen. Thus, the reservoir of the lumina appears to be filled with cellular material and has a cobweb-like structure in cross section. Protruding parts of the epithelial cells are dark coloured and contrast with the inner parts containing nuclei (Fig. 1F). Secretory and non-secretory areas are not distinguishable using light microscopy.

The length of the scent glands of the nemastomatid *Paranemastoma quadripunctatum* is about 6 to 9% of the body length. Scent gland openings located dorsal and near to coxae I are directed ventrally and again lead into an atrium. A lateral fold of the body and the dorsal parts of coxa I delineate the atrium leaving a rather large laterally directed opening (Fig. 1C). There are some cuticular spines on the dorsal side of the coxa but no compact lateral barrier of cuticular papillae. Anatomically, a single-layered epithelium surrounds a large cavity without any noticeable content. An intima that protrudes in many folds into the lumen of the scent gland covers the epithelial cells (Fig. 1D). No distinct secretory and non-secretory areas could be distinguished using light microscopy.

The length of the scent glands of the eupnoid *Amilenus aurantiacus* is about 6 to 10% of the body length. Slit-shaped scent gland openings are embedded in an elaborated pore structure that is exposed on a cuticular protrusion dorsal to legs I (Fig. 1A). One large cuticular spine is found in front of the pore. The pore with a crater-like rim is about 100 μm in diameter. Scent gland epithelial cells are very voluminous with large nuclei (Fig. 1B). Between the epithelial cells and intima there is many dark granules.

DISCUSSION

The present study indicates there are several distinct types of scent glands in soil-dwelling harvestmen. Scent glands and scent gland openings of the eupnoid species *Amilenus aurantiacus* are similar to those of *Leiobunum vittatum* (Say, 1821) and *L. flavum* Banks, 1984 (Clawson 1988). These are classified as *Leiobunum*-type, which possibly characterizes a part of the sclerosomatid Eupnoi. In contrast, all species of troguloid Dyspnoi investigated in this study share one common feature, namely a more or less developed construction of an external atrium that covers the ozopores. Due to the absence of a lateral wall of cuticular papillae, scent gland openings of the nemastomatid species *Paranemastoma quadripunctatum* are visible externally, whereas ozopores are completely hidden in species of Trogulidae. Furthermore, the structure of the scent glands of *P. quadripunctatum* indicates a defensive function since they resemble typical allomonal glands of, e.g., cyphophthalmid harvestmen and many other arthropods (e.g. Gutjahr et al. 2006). However, the atrium in trogulids is nearly closed, suggesting that defensive secretions would have to pass through the atrium and the narrow secondary opening before reaching the outside, and thus, a potential offender. Moreover, in both trogulids investigated there was solid material in the glandular lumina, which indicates they may not have a defensive function. However, in *Anelasmocephalus hadzii* parts of the epithelial cells in the scent glands seem to be vesicular constrictions of apocrine secreting cells while the spherical structures in the scent gland reservoirs of *Trogulus tricarinatus* probably are a solid secretion. Considering these rather untypical features, the defensive function of scent glands in Trogulidae is unlikely as was recently pointed out (Schaidler & Rasputnig 2009). As already mentioned, solid boli of secretion, similar to the “concretions” in *T. tricarinatus*, were also found in scent glands of some ischyropsalidids (also Dyspnoi), with the mode of release of the secretion so far remaining speculative (Juberthie et al. 1991, Gnaspini & Hara 2007). Glands in *Amilenus aurantiacus*, the only eupnoid species included in this study,

resemble more typical opilionid defensive glands: ozopores occur in an exposed position and based on preliminary studies, *A. aurantiacus* may release secretion from these pores forcefully in the form of a directed jet.

With respect to chemistry, the secretions of all the species investigated are poorly known and the scent gland chemistry of Dyspnoi is completely unknown, thus constituting a large gap in opilionid biology (Shear 2008). Only one pioneering study on the chemistry of the scent gland secretions in Dyspnoi (using the model of *Paranemastoma quadripunctatum*) has been completed, and it indicates unusual, naphthoquinone and anthraquinone-rich exudates (Raspotnig et al., unpublished).

In conclusion, among the Opiliones, especially the Palpatores, there is an obvious heterogeneity in scent gland characters with differentially developed “defensive” glands in Eupnoi and quite unusual scent glands in many Dyspnoi. In soil-living Dyspnoi, distinct evolutionary traits may have led to types of scent glands and functions other than chemical defence. With respect to the current discussions on the monophyly or paraphyly of Palpatores (Shultz & Regier 2001, Giribet et al. 2002), these features may reflect palpatorean evolutionary lineages and may be a source of novel data for tracing the phylogeny of Palpatores.

Acknowledgements

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