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Sexual dimorphism in venom gland morphology in a sexually stinging scorpion

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Males of several scorpion species possess bigger telsons than females. In at least some of these species, males repeatedly sting females during mating. This behaviour ('sexual sting') is likely correlated with a sexual dimorphism in telson and venom gland size. In sync with natural selection theory, females possess bigger venom glands because females need more nutrients for their offspring. Hence, we hypothesize that this sexual dimorphism in venom gland size evolved under sexual selection. We investigated the morphometrics and morphology of male and female telsons and venom glands of the sexually stinging scorpion *Euscorpius alpha* Caporiacco, 1950 (Euscorpiidae), using light and transmission electron microscopy. Male telsons are significantly bigger and more voluminous than those of females. Varying considerably between sexes, four different kinds of secretory cells are clearly distinguishable.The female secretory epithelium consists mainly of granule-filled cells while that of the males mainly has cells containing dissolvable vesicles. This cell type probably produces transparent venom that has been identified in other scorpions as so-called 'prevenom'. The role this "pre-venom" plays in sexual sting behaviour is addressed.

ADDITIONAL KEYWORDS: *Euscorpius alpha* – histology – scorpions – secretory cells – sexual selection – telson – venom variation

INTRODUCTION

Sexual dimorphism in scorpions occurs in several forms. Males and females may differ from each other in body size, shape of various body structures and the presence or absence of certain features. As in many invertebrates, female scorpions are generally larger or more robust than males (Polis & Sissom, 1990; Fox, Cooper & Hayes, 2015; but see Alexander, 1959). They feed proportionately more often than mature males as they require more prey to to feed themselves and their developing offspring (Polis & Farley, 1979a, b; Bradley, 1989; Polis & Sissom, 1990; Brown & Formanowicz, 1995; Outeda-Jorge, Mello & Pinto-da-Rocha, 2009; but see Bradley, 1984; Polis, 1986). However, despite the generally bigger body sizes of females, males of the genus Euscorpius bear (Kraepelin, 1908; Pavlovsky, 1913; Polis & Sissom, 1990; Fet *et al*., 2013). Sexual dimorphism is witnessed in several species whereby larger telson size, shape and structural elaboration is present in males but not in females (Polis & Sissom, 1990; Soleglad & Fet, 2004; Booncham *et al*., 2007; Lourenco & Duhem 2010). Some species of Bothriuridae, for example, have male caudal glands (Peretti, 1997; Olivero *et al*., 2015).

Scorpions show consistent sexual dimorphism in pectines, structures which are widely used by males during courtship and mating. Since they typically reach disproportionally larger sizes in males than in

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females, they are considered to represent secondary sexual traits in males evolved under sexual selection (Fox *et al.*, 2015). Therefore, to explain dimorphism in telson size, one should consider the role of sexual selection shaping the size or form of this structure in males. Indeed, the male telson is integral during courtship (Baerg, 1954; Angermann, 1955, 1957; Polis & Sissom, 1990; Benton, 1993; Peretti, 1997; Peretti & Carrera, 2005; Olivero *et al.*, 2015). In general, scorpion mating is a complex process involving several characteristic behaviours (Polis & Sissom, 1990). Typically, the male leads the female in a classical *promenade à deux* – he grasps the female's pedipalps in his own and guides the female to a suitable location for spermatophore deposition. During the promenade, the male engages in various activities such as sand scraping, juddering, cheliceral massage, telson rubbing, clubbing or sexual sting (Angermann, 1955, 1957; Alexander, 1959; Polis & Sissom, 1990; Benton, 1993). The male uses the metasoma and telson in the latter three behaviours. While rubbing the female's body with his telson, the male may produce secretions from his caudal glands, which may serve in intersexual chemical communication (Peretti, 1997; Olivero *et al.*, 2015). During clubbing, the male strikes the partner with the metasoma while concealing the stinger. On the other hand, the male sinks his stinger into the female's membranous articulations for several minutes during the sexual sting.

Sexual sting was recorded in representatives of the families Bothriuridae, Euscorpiidae (incl. genus *Euscorpius*), Chactidae, Iuridae, Scorpionidae and Vaejovidae (Francke, 1979; Polis & Sissom, 1990; Peretti, Acosta & Martínez, 2000; Tallarovic, Melville & Brownell, 2000; Booncham *et al.*, 2007; Jiao & Zhu, 2010; Toscano-Gadea, 2010). Despite its uniqueness and peculiarity, little attention has been given to this behaviour in the literature nor has it been investigated in detail. Although sexual sting is considered to be a tool a strategy to deescalate lower female aggressive behaviour or to succumb her for copulation, (Polis & Sissom, 1990), it might also represent a stimulating impulse (Inceoglu *et al.*, 2003). It is not known whether venom is transferred during these stings or whether it is just a mechanical stimulus. Moreover, even though it might seem that the male stings the female, his stinger does not necessarily pierce her body (Peretti, 2014).

There is no study linking sexual dimorphism in telson size and sexual sting in scorpions. The co-occurrence in the genus *Euscorpius* lead us to hypothesise that males are probably sexually selected to have larger telsons to produce a substance in greater concentration or composition than produced in females. It is assumable that this substance is transferred during sexual sting to the female body. To test this hypothesis morphologically, we inspected the content of the telson of *Euscorpius alpha* Caporiacco, 1950, a scorpion species in which males sting females during courtship (Kropf C, personal observation; Braunwalder, 2005 for genus *Euscorpius*) to determine whether the glandular apparatus differs between sexes (i.e. males possess unique secretory cells that are not present in females) or whether the difference is simply in size and/or in the number of secretory cells. This study presents the first comparison of male and female venom gland morphology in a sexually dimorphic scorpion and its potential relationship to the sexual sting.

MATERIAL AND METHODS

Adult males (N = 5) and females (N = 5) of the scorpion *E. alpha* were collected in Mendrisiotto district in the Swiss Canton of Ticino Ticino, during the months of June and August 2014. They were placed in transparent containers and fed crickets on the first day of captivity. After one week, the individuals were anesthetized with ether and processed accordingly (see below).

LIGHT MICROSCOPY

For the histological study, six telsons (three from each sex) of scorpions captured in July were inspected. The telson was removed with fine surgical scissors. The tip of the telson (i.e. aculeus or stinger) was removed to enable penetration of the fixative to the telson. The samples were fixed in 2.5% glutaraldehyde in Hepes buffer (0.15 M, pH 7.34) and then subjected to post-fixation in buffered 1% osmium tetroxide. The samples were afterwards washed in maleate buffer, dehydrated in graded ethanols and embedded in Epon.

Due to the incomplete penetration of the fixative with the previous procedure, four additional individuals (two females and two males) were collected on the location mentioned above in August 2014 and they were kept and anesthetized as described above. Their telsons were removed with fine surgical scissors. To enable better penetration of the fixative to the glands, a tip of the telson was removed and the rest of the telson was cut longitudinally in half with a razor blade. It was unavoidable to squeeze the metasoma a little, so a small clear droplet came out of the stinger, its size appearing uniform for both males and females. The proportional representation of empty cells did not differ between the sexes [linear mixed effect (LME) model, $F_{2,168} = 4.2$, $P = 0.2$], so it is unlikely that females lost significantly more venom than males during this procedure. Both halves were fixed in 2.5% glutaraldehyde in phosphate buffer (0.1 M, pH 7.4, 1.8% sucrose) and then subjected to

postfixation in buffered 1% osmium tetroxide. The samples were afterwards washed in sodium cacodylate buffer, dehydrated in graded ethanols and embedded in Agar Low Viscosity Resin.

Serial semi-thin sections (1 µm, cross-sections) of male and female telsons were obtained using a Diatome Histo Jumbo diamond knife on a Reichert Jung Ultracut microtome. The sections were placed on glass slides, heated and stained with 1% toluidine blue. Examination of the sections was performed with a Zeiss Axioplan 2 microscope connected to a Zeiss Axiocam MCR digital camera. Different types of gland cells were distinguishable already under the light microscope and their number was recorded for one of the paired glands on approximately every 20th section. Only sections produced from Agar Low Viscosity Resin blocks were used for the quantitative analyses. On average, 49 sections were inspected for each male (N = 2) and 37 for each female (N = 2). So far, this method is unique to our study.

The relative number of each cell type per section was calculated by diving the number of gland cells of a given type by the total number of gland cells per section. It was transformed using angular transformation to homogenize the variances. In order to compare the relative number of cell types between sexes, a LME model was ran for each of the four cell types. The relative number of the given cell type was set as a response variable and sex as the explanatory variable. Individual and section were added to the model as random effects. To compare the total number of cells per section between sexes, an additional model was run in the same way as the four previous models but with the total number of cells per section as dependent variables.

Transmission electron microscopy

After each 40th semi-thin section, ultrathin sections (60 nm, cross-sections) were obtained from the same blocks described above using a Diatome Ultra 45° diamond knife on a Reichert Jung Ultracut microtome and placed on copper slot grids. Post-processing included staining with saturated uranyl acetate and lead citrate. The sections were examined with a transmission electron microscope (TEM) Philips EM 400 in order to reveal ultrastructural details of the venom gland (these sections were not used for the count of gland cell types). Images were obtained with a Morada 12 Megapixel camera using AnalySIS ITEM software.

Measurements

Length, width and height of telsons of additional adult males (N = 11) and females (N = 15) from the same population from the Natural History Museum, Bern collection were measured using a Keyence VHX-2000 digital microscope. The measurements were compared using a Welch two-sample t-test with Bonferroni correction. In addition, width of fifth metasomal segment was also recorded to control for the possibility that telson size varies with overall body size (i.e. male telsons are bigger simply because males are bigger).

RESULTS

Overall appearance of the venom gland

The venom apparatus of *E. alpha* is composed of a pair of venom glands situated in the telson (Fig. 1). Each gland is partly covered by cuticle and muscles whereby the gland itself shows no folding. The glandular cells are of equal length and more or less alike in height throughout the gland (Fig. 1) which are features characteristic for simple type glands (Type 1) according to Pavlovsky (1913, 1924). The glandular tissue is composed of secretory cells and non-secretory supportive cells. The secretory cells are filled with different vesicles which are released into a narrow, centrally located reservoir. The glands run bilaterally through the whole telson length. Distally, they narrow into two separate venom ducts which open to the outside just beneath the tip of the aculeus.

Muscles and connective tissues

Each gland is fixed to 1/3 (close to the tip) or 1/2 (more proximally in the telson) at the cuticle; the rest is surrounded by a thick girdle of striated muscle (Fig. 1). The muscles occur in several bundles (five to nine) which can be separated by thin unicellular layers of connective tissue containing numerous vesicles (Fig. 2A). The actin-free H zone and its central part, the M zone, are indistinguishable while the Z lines (i.e. the borders of the sarcomeres) show irregular structure. Between muscle and mitochondria, rows of glycogen granules can be found. The muscle fibrils attach to the cuticle in numerous branches by tendon cells (Fig. 2B). These tendon cells are attached to outgrowths of the cuticle (Fig. 2B, C). The muscles and the cuticle are separated from the glandular epithelium by a thick membrane formed by supportive cells (see below, Fig. 2D). Between the membrane and the gland cells, a row of voluminous cells is found (Fig. 2D). The cuticle and muscles adjoin the membrane formed by supportive cells. However, there may also be cells containing vesicles located in between (Fig. 2D, E). Nerves flank both sides of this membrane (Fig. 2F). Sometimes proliferations of the membranes towards both sides can be seen (Fig. 2E), leading to a tighter connection between the

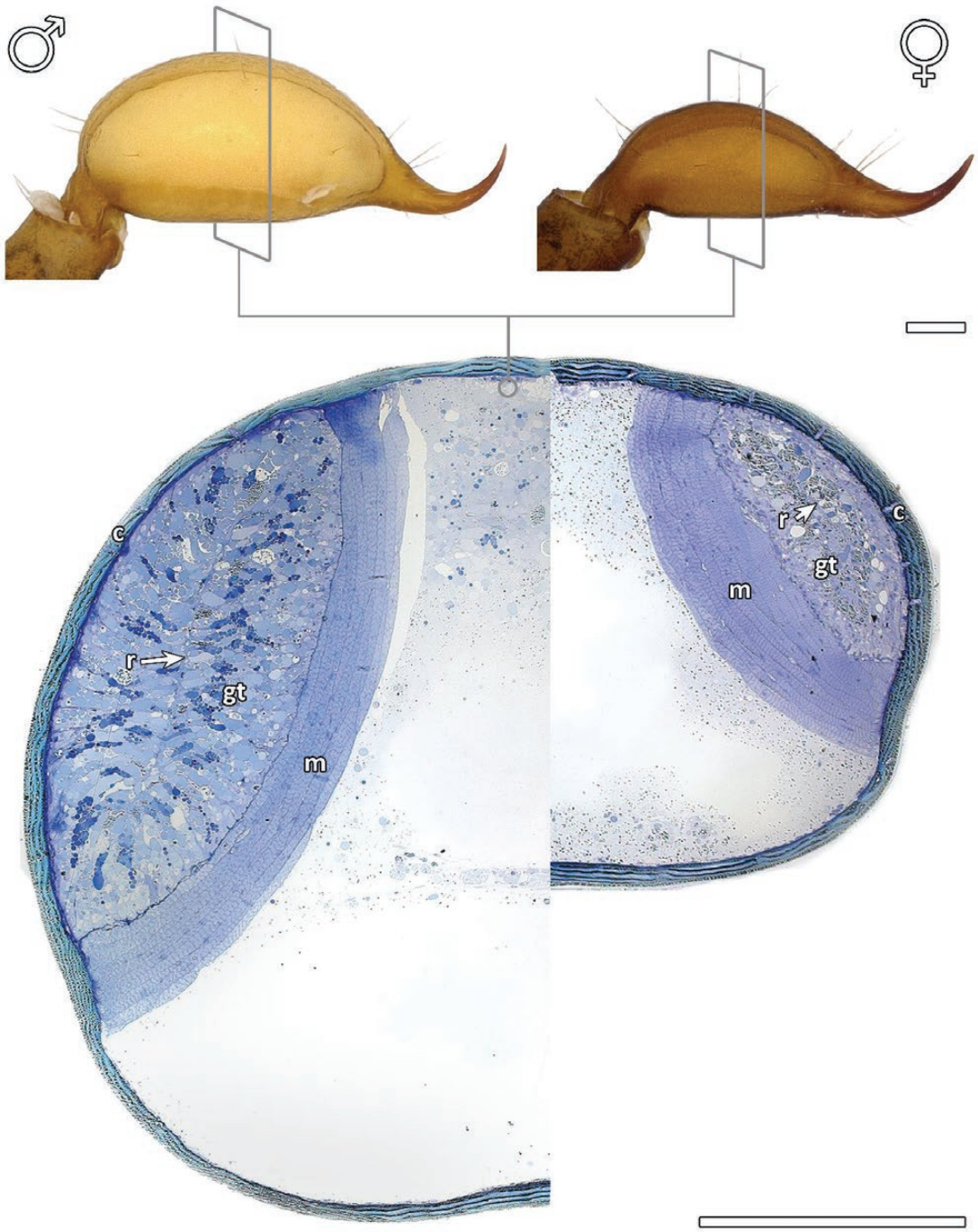

Figure 1. External and internal (semi-thin cross-section) morphology of male and female telson of *Euscorpius alpha* showing size differences between the sexes. A simple (Type 1) paired venom gland runs longitudinally through the telson of both sexes. cu, cuticle; gt, glandular tissue; m, muscles; r, reservoir. Scale bar = 500 µm.

membrane and the adjacent tissues (muscles, connective tissue cells, epidermal cells). The connective tissue cells have complex folded cell membranes, pointing to a high transport activity.

Secretory cells

The secretory cells are columnar and show cell membranes with septate desmosomes (Fig. 3A), forming ladder- or grid-like structures (Fig. 3A). The nucleus

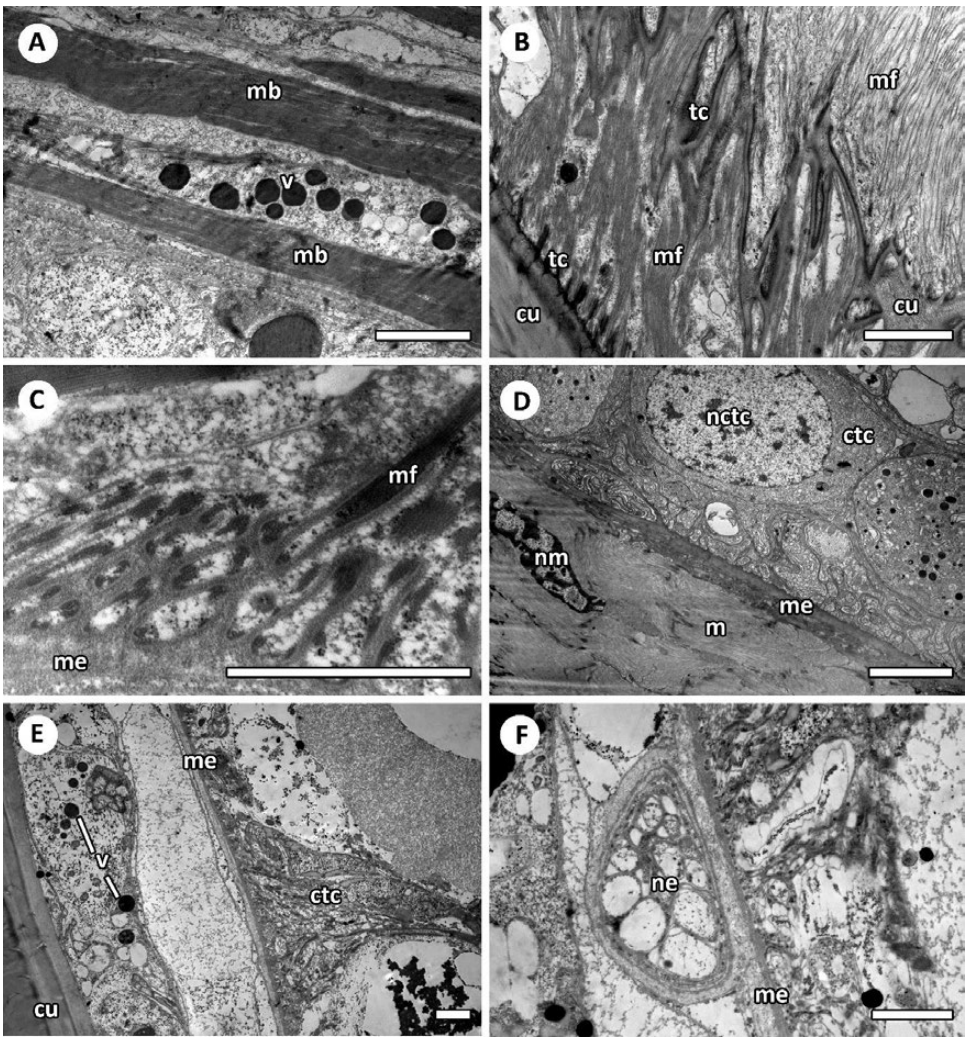

Figure 2. Muscles and connective tissue surrounding the glandular tissue of the venom gland of *Euscorpius alpha*. (A) Muscle fibrils organized in muscle bundles (mb) separated by connective tissue with vesicles (v). (B) Attachment of the muscle fibrils (mf) to the tendon cells (tc). (C) Attachment of the muscle fibrils to the membrane of supportive cells. (D) Thick membrane of supportive cells adjoining directly to the muscle and to a row of voluminous connective tissue cells. (E) Same membrane separated from the cuticle by vesicle-containing cells. (F) Nerve associated with the thick membrane of supportive cells. cu, cuticle; ctc, connective tissue cell; m, muscle; mb, muscle bundle; me, membrane formed by supportive cells; mf, muscle fibrils; nctc, nucleus of a connective tissue cell; ne, nerve; nm, muscle nucleus; (tc), tendon cells; v, vesicle. Scale bar = 2 μm.

and densely packed endoplasmic reticulum (ER) are in the peripheral region of the basal part of the cell (Fig. 3B). Mitochondria are interspersed throughout the ER and probably serve as a cell 'factory' to produce the vesicles. Cells containing largely endoplasmic reticulum but very few to no vesicles, are likely empty cells. However, most of the cell is filled by vesicles of different size, shape and electron density. Four distinct secretory cell types can be distinguished with both light and TEM according to the vesicles they contain:

Type A cells are filled with rather large circular vesicles (Figs 3A, 4A). The diameter of the vesicles exceeds 3 μm and reaches up to 10 μm. The vesicles occur in two forms whereby, both show the same size range but demonstrate different degrees of electron density. Some appear homogeneous while others appear highly granulated. In some cells, both vesicle types occur while transitions between the two can be seen (Figs 3B, 4A). In addition, a very similar (if not identical), finely grained substance observed in some vesicles is also found in the cytoplasm of cell type A as

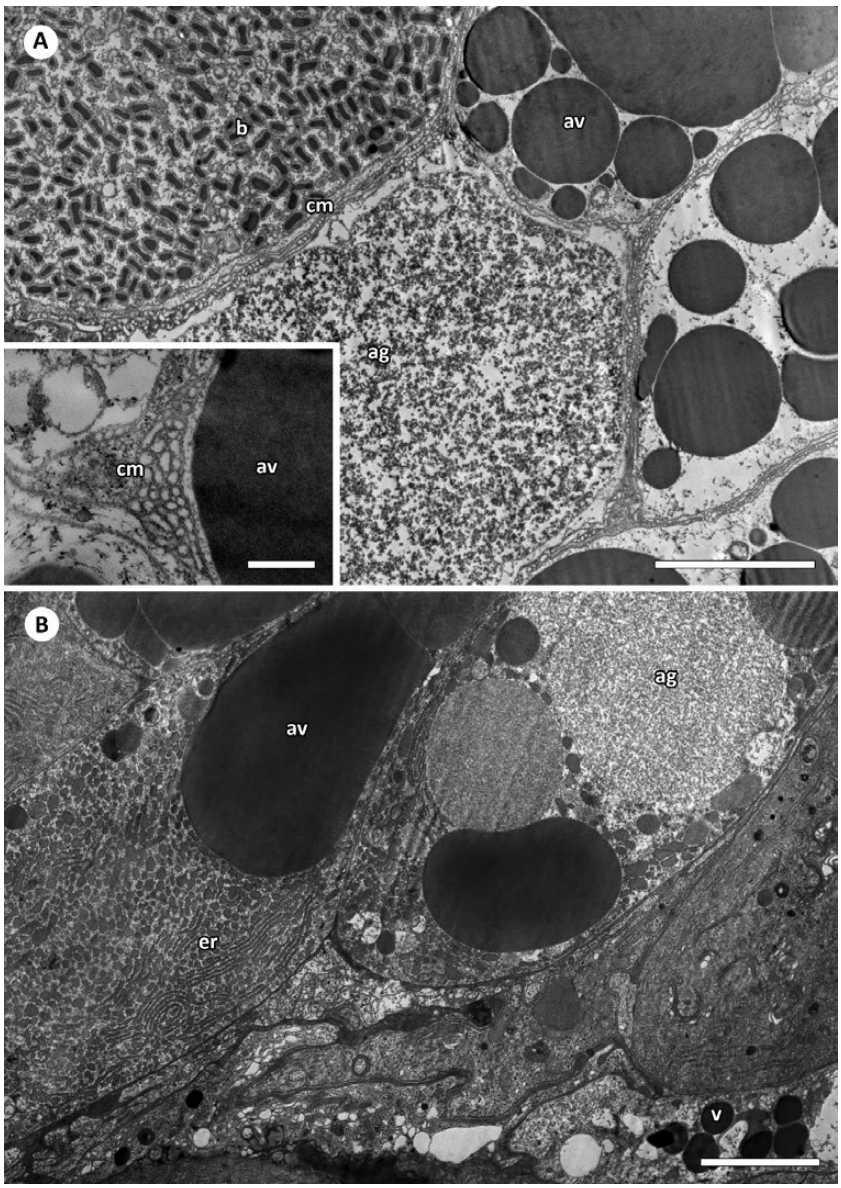

Figure 3. Glandular tissue of the venom gland of *Euscorpius alpha*. (A) Glandular cells filled by different vesicles separated by cell membranes with septate desmosomes. The inset shows detail of a septate desmosome forming a grid-like structure. (B) Basally located factory part of glandular cells with ER and few vesicles (different stages of Type A cells). ag, grained substance of Type A cells; av, vesicles of Type A cells; b, Type B cells; cm, cell membrane; er, endoplasmatic reticulum; v, vesicles. Scale bar = 5 μm, inset = 500 nm.

well as in the reservoir. Therefore, we conclude that the content of the vesicles develops from a homogeneous state towards a finely granulated one, forming a coarse to fine-grained substance (Figs 3B, 4A) which can also be found in the cytoplasm and the reservoir. Sometimes, the cell filled by this substance also contains one or a few large vesicles as described above.

Type B cells contain small (max. 1 μm long), electron-dense oval, barbell- or kidney-shaped vesicles (Fig. 4B). Within the cell, some of the vesicles form an aggregate surrounded by a membrane, but most of them exist freely in the plasma.

Type C cells are irregularly shaped and filled with medium-sized (1–3 μm) electron-dense vesicles (Fig. 4C). The vesicles are smaller and less round those of type A and bigger but never kidney-shaped as in type B.

Type D cells are typically formed by a row of compartments with electron-dense droplets in the centre, separated by thin thread-like 'bridges' (Fig. 4D). The droplets show no membranous cover.

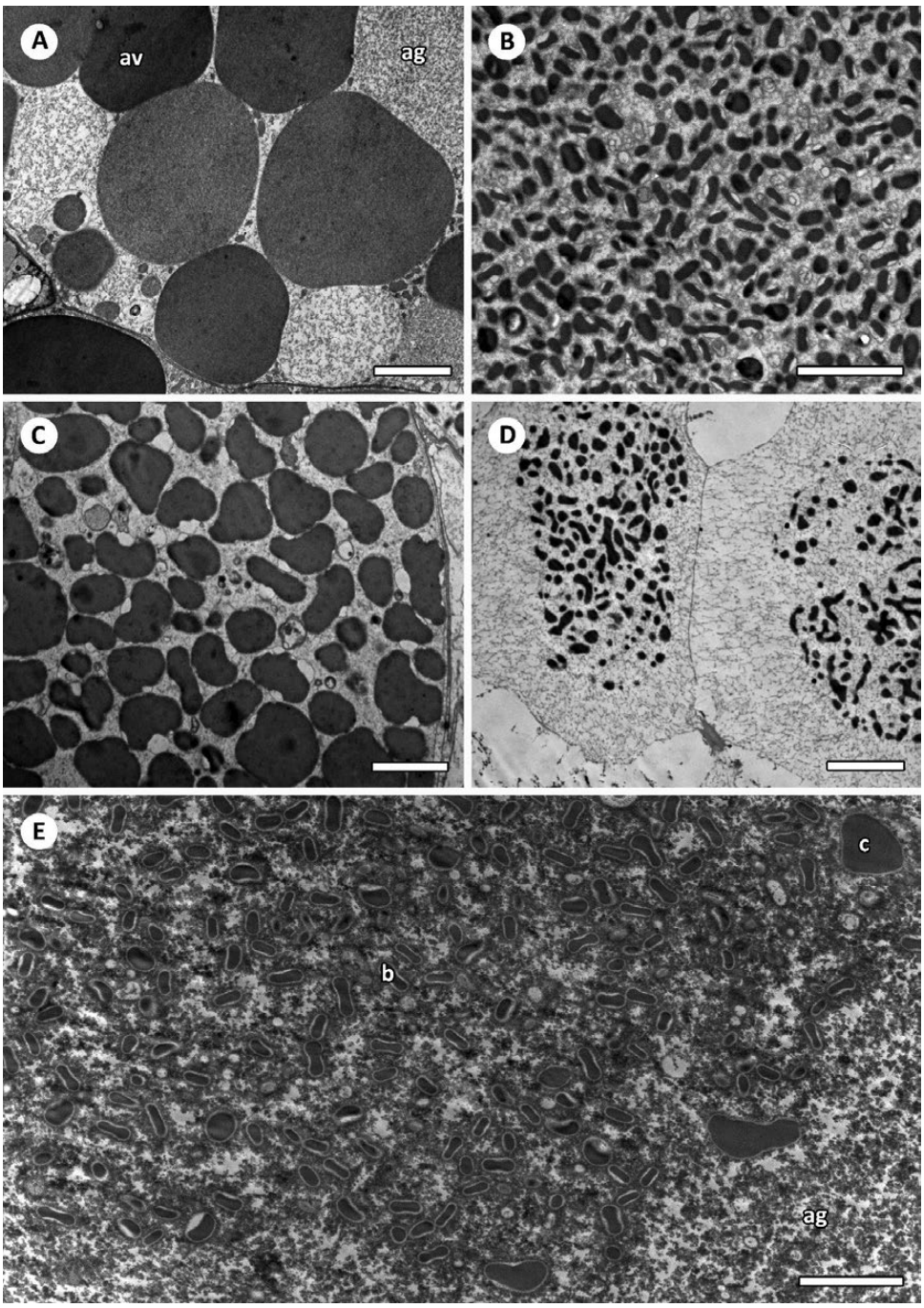

Figure 4. Four types of glandular cells of the venom gland of *Euscorpius alpha*. (A) Type A cell filled by circular vesicles and grained substance. (B) Type B cells containing small oval vesicles. (C) Type C cells filled by irregular vesicles of medium size. (D) Type D cells formed by a row of compartments with electron-dense droplets in the centre. (E) Centrally located reservoir of the venom gland filled by vesicles of Type A (grained substance), B and C. Scale bar = 3 μm.

The fine-grained substance encountered in the A cells and the vesicles of type B and C cells was also found in the reservoir (Fig. 4E). Rarely are the homogeneous, electron-dense vesicles of cell type A found in the reservoir. However, this could be an artefact related to squeezing while opening the telson for fixative penetration.

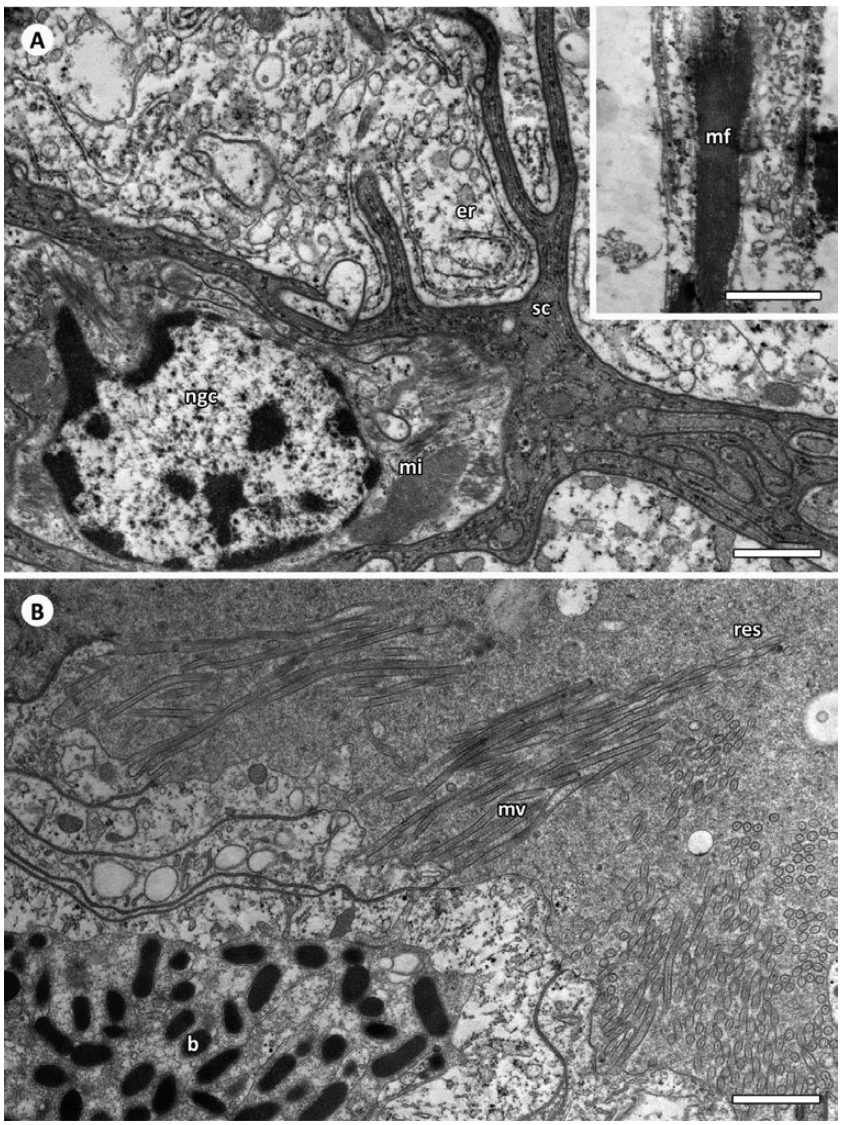

Figure 5. Supportive cells within the venom gland of *Euscorpius alpha*. (A) Basal part of a supportive cell branching between factory parts of glandular cells. The supportive cell is packed with granules and membranes; inset shows muscle fibrils within the cell. (B) Microvilli penetrating glandular reservoir. b, Type B cell; er, endoplasmatic reticulum; mf, muscle fibrils; mi, mitochondria; mv, microvilli; ngc, nucleus of a gland cell; res, reservoir; sc, supportive cell. Scale bar = 1 µm.

Supportive cells

An extensive net of long, slender, electron-dense supportive cells is located between the secretory cells (Fig. 5A). Muscle fibrils are present within the matrix of supportive cells, which form the thick (Fig. 5A). The cells form the thick membrane surrounding the glandular epithelium. Proliferations of this membrane may be leading in all directions. The nucleus is close to the membrane at the basal region of the cell. The supportive cells expand towards the reservoir and cover the gland cells in a grid-like matrix. These cell expansions are packed full of granules of different size and electron density bearing numerous tubes and double or multiple membranes (Fig. 5A). The cells branch into numerous slim processes that again split into microvilli containing microfilaments and penetrating into the reservoir (Fig. 5B). These cell expansions surround large parts of the gland cells where they open into the reservoir. The microvilli of the supportive cells are most dense at the periphery and the lowest at the centre of the reservoir the centre of the reservoir. Interestingly, the tubes within the cell expansions look identical to the microvilli and also contain microfilaments.

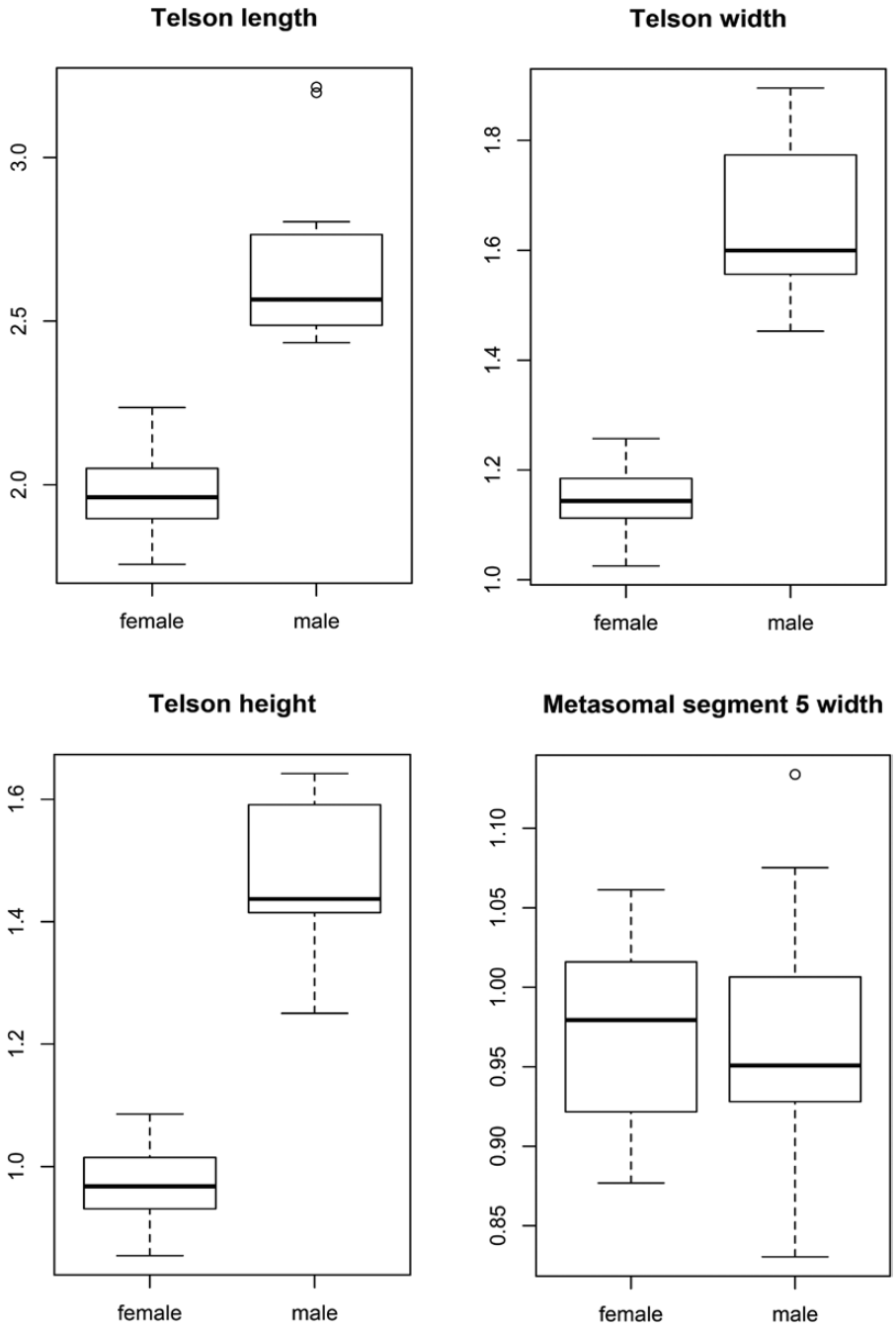

Figure 6. Comparison of male and female telsons in *Euscorpius alpha*.

Comparison of male and female venom apparatus

Adult male telsons in *E. alpha* are considerably bigger and more voluminous in comparison to the telsons of the adult females (Fig. 6; ♂ N = 11, width = 1.45–1.90 mm, height = 1.25–1.64 mm, length = 2.43–3.22 mm; ♀ N = 15, width = 1.03–1.26 mm, height = 0.85–1.09 mm, length = 1.76–2.24 mm). All differences were highly significant for the telson measurements (Welch two-sample t-test, $P < 0.001$) except the width of the fifth metasomal segment which did not differ between sexes (Welch two-sample t-test, $P = 0.983$).

Several differences between sexes are encountered at the cellular level (Table 1). The male glands contain nearly three times more secretory cells than the female glands. Three of the four cell types differ between sexes. Type A cells are significantly more abundant in males than in females. In females, these

Table 1. Differences in the cell equipment of the venom gland in males and females of *Euscorpius alpha*

Cell type	Male (N = 2)		Female (N = 2)		LME	
	Count ± SD	%	Count ± SD	%	$F_{2,168}$	P
A	3534 ± 659	88.3	339 ± 86.3	23.6	37.3	0.025
B	172 ± 66.5	4.3	384 ± 41	26.7	81.2	0.012
C	235 ± 164	5.9	701 ± 143.5	48.7	33.1	0.03
D	62 ± 11.3	1.5	14 ± 7.1	1.0	8.7	0.1
Total	4003 ± 439.8		1438 ± 263.8		21.6	0.04

The average numbers of gland cell types within the gland per individual and their proportional representations are given. Linear mixed effect (LME) model statistics show differences between sexes in the relative number of gland cell types and in the total number of gland cells.

cells are situated mainly close to the tip of the telson and are nearly absent at the more proximal region of the gland. In males, this cell type is the most abundant throughout the whole gland and is the most common of all secretory cells. Type B cells occur throughout the glands of both sexes but are significantly more abundant in females. The same holds true for type C cells, which are the most abundant cell type in females, comprising nearly half of all secretory cells present in the gland. Type D cells occur with similarly low abundance in both sexes and represent the rarest cell type.

After complete fixative penetration in glands prepared in Low Viscosity Agar Resin, cells were counted throughout the entire gland length. Despite incomplete fixation by EPON, the glands illustrated the same cellular patterns between males and females as described above. We did not include the EPON sections into the statistical analyses as the telsons were fixed and processed differently.

DISCUSSION

Although venom glands of several scorpion species have been examined ultrastructurally (Kubota, 1918; Rosin, 1965; Keegan & Lockwood, 1971; Mazurkiewicz & Bertke, 1972; Kovoor, 1973; Yahel-Niv & Zlotkin, 1979; Halse *et al*., 1980; Kanwar, Sharma & Nagpal, 1981; Kanwar & Nagpal, 1983; Taib & Jarrar, 1993; Yigit & Benli, 2007, 2008, 2009, 2010; Jarrar & Al-Rowaily, 2008), none of these studies provides a comparative description of male and female glands nor states whether intersexual differences occur.

Sexual dimorphism in *E. alpha* was found in telson and venom gland size, secretory cell number and their proportional representation. Although females are known to possess larger telsons in other scorpions such as the bothriurids (e.g. Ojanguren-Affilastro 2007), measurements confirmed that males possess significantly bigger telsons harbouring more secretory cells than females in *E. alpha*.

Braunwalder (2005) found that body size and sexual body size dimorphism of *E. alpha* vary considerably between populations. He measured 49 females and 24 males from the population from which the scorpions used for this study derive, and found the female body to be only 0.8 mm longer than the average. When pooling these data with data from three additional populations (n females = 157; n males = 83 in total), this size difference of 0.8 mm remained. In addition, Braunwalder (2005) reported and figured a sexual telson size dimorphism in *E. alpha* with males showing considerably bigger telsons than females. Our data on telson length, width and height strongly support Braunwalder's (2005) conclusions. However, due to low numbers of available specimens and the fact that both sexes may become mature either in the fourth or fifth instar (males) and fifth or sixth instar (females), a full statistical description of this dimorphism cannot be presented here but remains for future studies based on a larger sample size.

Braunwalder (2005) described a common scheme of successive mating behavioural patterns, including sexual sting, in all *Euscorpius* species observed so far. Amongst these, he reported also three matings of *E. alpha* (Braunwalder, 2005). This is congruent with our anecdotal observation of mating behaviour, including the sexual sting in this species. Considering the behaviour of 'sexual sting' in this species, we assume that the sexual dimorphism in venom gland size in *E. alpha* is related to sexual selection.

The male gland is equipped mainly with type A cells. Although they comprise nearly 90% of male secretory equipment, they cover less than 25% of secretory cells in females. Based on the numerous transitional stages (Figs 3B, 4A; homogenous dark vesicles – finely granulated lighter vesicles – finely granulated light substance filling whole cell), we consider the high electron dense, homogenous granules as the early stages within type A cells, which gradually dissolve and unite in a big mass of finely granulated substance. They fill not only some of the cells but also a large

proportion of the reservoir as well. Large electron-dense vesicles were only rarely found in the reservoir. Cells filled by the finely granulated substance have often been described often described in the literature as mucus cells (Kubota, 1918; Keegan & Lockwood, 1971; Kanwar & Nagpal, 1983; Farley, 1999), whereby Keegan & Lockwood (1971) pointed out that the content of these cells is not entirely typical of mucus cells found elsewhere.

The female secretory epithelium is mainly composed of cells containing granules. In general, granules are considered to represent venom proteins (Kubota, 1918; Keegan & Lockwood, 1971; Farley, 1999). Inceoglu *et al.* (2003) distinguished two types of venoms – prevenom with a low concentration of proteins and high concentration of K^+ salts and a protein-rich venom. Based on this study, our observation indicates that females of *E. alpha* possess mainly venom-producing cells, while males show lots of prevenom cells (probably erroneously termed 'mucus cells', see above) and much less venom-producing ones.

Type A cells are abundant in male venom glands and contain no granules. This finding corresponds to the so-called, 'prevenom' that Inceoglu *et al.* (2003) described for the buthid *Parabuthus transvaalicus*. Inceoglu *et al.* (2003) speculated that it is this prevenom that is transferred into females during sexual sting. Given the effects of the prevenom, its use during mating is conceivable (however, data are entirely missing) as it may modify muscular contractions of the female during the promenade without exposing her to venom components potentially toxic to her (Inceoglu *et al.*, 2003). However, in *P. transvaalicus*, no sexual sting has yet been observed. In females of *E. alpha*, type A cells are only found distally in the gland, that is, near the base of the stinger. This instance could point to an initial release of type A content and a subsequent release of the other cell contents, potentially corresponding to prevenom and venom. Moreover, it could well be that type A cells produce a prevenom in *Euscorpius* as well and that it is plausible that it is transferred during sexual sting. However, as *E. alpha* and *P. transvaalicus* are two non-related species (belonging to different families), this problem clearly needs further investigation to clarify whether prevenom indeed exists or is used at all this species.

Prevenom is typically produced only in small quantities (Inceoglu *et al.*, 2003) and so far, there are no data on intersexual differences in its production. Furthermore, although we found sexual dimorphism in gland size and its cell composition, further clarification is necessary as to whether males of *E. alpha* produce different venom than females. It has been shown that venom composition in venom-producing animals may vary at the individual, geographic or species levels (e.g. El Ayeb & Rochat, 1985; Abdel-Rahman *et al.*, 2011; Sunagar *et al.*, 2014) and that males and females may be sexually dimorphic in venom composition (e.g. D'Suze, Sandoval & Sevcik, 2015; de Sousa *et al.*, 2010; Rodríguez-Ravelo *et al.*, 2015). In *Scorpio maurus palmatus*, the female venom contains more components (Abdel-Rahman *et al.*, 2009) while in the spider *Tegenaria agrestis*, both sexes possess the same components but the venom composition differs between sexes in their relative abundance (Binford, 2001). Current research of venom variation focuses on proteomic and/or transcriptomic variation while histological identification of differences in glandular architecture is mostly lacking. However, if different glandular cell types can be linked to specific venom proteins, their proportional representation in the gland might correlate with venom composition. On the other hand, the venom composition may still differ even though the morphology shows the same cell types. For example, two scorpion species of the genus *Centruroides,* which differ greatly in their toxicity, show the same gland morphology (Keegan & Lockwood, 1971).

Sexual dimorphism in telson size and venom gland morphology may be a result of sexual selection related to sexual sting. However, it may also arise from different lifestyles of males and females where different selective pressures may act upon the sexes. While females tend to be sedentary, males become vagrant during the mating period and thus, they are more exposed to predators during this time (Polis & Farley, 1979b; Polis & Sissom, 1990; Benton, 1992). Bigger telsons might be favoured in males because they are exposed to higher predation pressure and therefore, need more venom for defence (but see Miller *et al.*, 2016). Despite these considerations, it is unlikely that these reflect the actual situation since male vagrancy is widespread amongst scorpions and there are only few species where males possess bigger telsons. (Table 2). Similar ambiguity holds for sexual dimorphism in pedipalps and chelicerae in scorpions (Carrera, Mattoni & Peretti, 2009). Intersexual differences in these structures might be explained by their usage during mating as well as to the differences in life style of males and females (e.g. burrowing behaviour, Polis, 1990; Prendini, 2001). There is, however, a strong correlation between sexual dimorphism in chelicerae and the occurrence of the chelicerae grip during mating (Carrera *et al.*, 2009). So far, sexual sting has been observed in scorpion species across eight families (Table 2) but reports about sexual dimorphism in telson size are mostly lacking. Therefore, a morphological and behavioural survey across scorpion taxa would be necessary to shed light on sexual dimorphism in the venom gland and its relation to sexual sting.

Table 2. Compilation of information available on the occurrence of sexual dimorphism and sexual sting in scorpions

Species	Sexual sting	Telson dimorphism	Reference
Bothriuridae			
Bothriurus buecherli	Yes	?	Toscano-Gadea (2010)
Bothriurus cordubensis	Yes	?	Peretti *et al.* (2000)
Bothriurus noa	Yes	?	Peretti *et al.* (2000)
Bothriurus prospicuus	Yes	?	Peretti *et al.* (2000)
Urophonius brachycentrus	Yes	?	Polis (1990)
Buthidae			
Centruroides vittatus	?	Yes (different shape)	Polis (1990)
Caraboctonidae			
Hadrurus arizonensis	Yes	?	Tallarovic *et al.* (2000)
Chactidae			
Uroctonus spp.	?	Yes (male telson swollen, vesicle-aculeus juncture very distinct)	Soleglad & Fet (2004)
Anuroctonus spp.	?	Yes (highly swollen vesicle and aculeus base)	Soleglad & Fet (2004)
Anuroctonus phaidocatylus	?	Yes (swelling at the base of aculeus in males)	Polis (1990)
Chaerilidae			
Chaerilus pictus	?	Yes (male telson prominently elongated)	Lourenco & Duhem (2010)
Euscorpiidae			
Euscorpius alpha	Yes	Yes	This study
Euscorpius candiota	?	Yes	Fet *et al.* (2013)
Euscorpius carpathicus	Yes	?	Polis (1990)
Euscorpius flavicaudis	Yes	?	Polis (1990)
Euscorpius italicus	Yes	?	Polis (1990)
Euscorpius ossae	?	Yes	Fet *et al.* (2013)
Euscorpius scaber	?	Yes	Fet *et al.* (2013)
Megacormus gertschi	Yes	?	Francke (1979)
Scorpiops luridus	Yes (during initial phase)	?	Jiao & Zhu (2010)
Hemiscorpiidae			
Hemiscorpius sp.	?	Yes (male telson elongated and bilobed distally)	Polis (1990)
Scorpionidae			
Pandinus imperator	Yes	?	Garnier & Stockman (1972)
Heteromerus laoticus	Yes	Yes (males wider and longer telson)	Booncham *et al.* (2007)
Vaejovidae			
Paruroctonus mesaensis	Yes (during escape behaviour)	?	Polis & Farley (1979b)
Vaejovis carolinianus	Yes	?	Polis (1990)

Question marks indicate no information available.

An elaborated system of supportive cells, their processes and microvilli corroborated findings previously described for both sexes (Keegan & Lockwood, 1971; Yigit & Benli, 2007). The observed muscle fibres within the processes of the supportive cells suggest that they are mobile. Our observation that masses of 'intracellular' tubes look exactly like the microvilli could support an extreme mobility of these cells whereby, the

microvilli could be expelled or retracted depending on the reservoir volume. The microvilli also point to a high degree of material exchange between the reservoir and the supportive cells. Another possible function of the supportive cells is their potential role in controlling the extrusion of the gland cell products.

We found considerable intersexual differences in venom gland morphology. Although we cannot generalize that sexual dimorphism in the venom gland is exclusively related to sexual sting behaviour, we hypothesize that males possess bigger venom glands in order to produce a substance employed during sexual sting. The most abundant type of secretory cells in males do not contain granules (considered to represent proteins). Inceoglu *et al*. (2003) identified a clear, protein-poor substance as prevenom and predicted its use during sexual sting. It is possible that the type A cells we recorded in *E. alpha* produce a prevenom and that this substance is transferred during sexual stinging. Therefore, we hypothesize that increased number of cell types B and C in females (that presumably contain the venom proteins) was shaped under natural selection, while the more numerous type A cells in males (that presumably produce the prevenom) is related to sexual selection and specifically, to the behaviour of 'sexual stinging'. Further investigation, including characterization of venom composition, yield and toxicity in conjunction with behavioural experiments, need to be conducted in order to test further this hypothesis and to confirm the link between histology and function.

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