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Female genital morphology and sperm storage in the velvet spider *Eresus kollari* (Araneae: Eresidae)

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Abstract. In the present study, we examined the female genital system of a velvet spider (*Eresus kollari* Rossi 1846) using light and electron microscopy. The female entelegyne genitalia of *E. kollari* comprises an epigyne with an anterior wide longitudinal bar and folds which are incurvated sidewards. The anterior end of these folds corresponds to enlarged anterior bulges which are connected to a distinct copulatory duct leading to lobular spermathecae. The anterior bulge is equipped with many large pores whereas the spermathecae has many small pores. At present, only a few studies have focussed on the ultrastructure and possible function of adjacent epithelia in entelegyne genitalia of spiders revealing the presence of complex class 3 gland cell units around the spermathecae and ducts. Alternatively our analysis finds two different types of epithelia. The anterior bulge is equipped with class 3 gland cells whereas the spermathecae are surrounded by a putative transport epithelium. This epithelium is characterized by an extensive basal labyrinth, numerous mitochondria, and an invaginated cell apex with microvilli. The functions of the different parts are unclear, but the secretion produced by the class 3 cell glands in the anterior bulge could be involved in the transport of sperm by flushing a considerable quantity of secretion towards the posterior. Alternatively, it could also contribute to the amorphous mass which is formed during mating covering most of the epigyne. On the other hand, the epithelium around the spermathecae might only be involved in the alteration of the milieu in the spermathecal lumen but not contribute to the nutrition of spermatozoa during sperm storage.

Keywords: Spermatheca, copulatory duct, glandular unit, spermatozoa

In spiders, females often mate with more than one male and sperm is often stored in the sperm storage organs (usually spermathecae) until fertilization takes place (e.g., Schneider & Andrade 2011). The sperm storage organs are distinct sclerotized areas equipped with a glandular epithelium consisting of glandular units comprised of several cell types and a distinct canal for discharging glandular products—class 3 gland cell units (e.g., Suhm & Alberti 1993, 1996; Uhl 1994a, 2000; Michalik et al. 2005). Moreover, glands can also be present around the ducts that lead to the spermathecae (e.g., Ramírez 2014). However, the function of the glandular products is not known. It was suggested that the secretion plays a role in nutrition and activation of spermatozoa (e.g., Herberstein et al. 2011; Vöcking et al. 2013).

In general, two different types of female genitalia are known for spiders—haplogyne and entelegyne. In haplogyne genitalia the copulatory duct also serves as the fertilization duct whereas in entelegyne genitalia distinct copulatory and fertilization ducts as well as a sclerotized plate (epigyne) are present (e.g., Uhl 2002). This fundamental difference led to the division of Araneomorphae into Haplogynae and Entelegynae (Simon 1892–1903), a division supported by several phylogenetic studies (e.g. Platnick et al. 1991; Griswold et al. 1999, 2005; Ramírez 2000).

Phylogenetically the most basal group of entelegynous spiders are velvet spiders (Eresidae) (summary in Miller et al. 2012). They are a small spider family with 96 species. With one exception, they are restricted to the Old World and live as sit-and-wait predators. Prominent taxa including the genus *Stegodyphus* Simon 1873, which shows various degrees of sociality (e.g., Kraus & Kraus 1988; Johannesen et al. 2007), and the European species of the genus *Eresus* Walckenaer 1805, named ladybird spiders due to the eye-catching coloration of the male opisthosoma. Considering their phylogenetic position within Entelegynae, Eresidae could be crucial for the understanding of the evolution of entelegynous genitalia. Their female genitalia are conspicuous as no distinct copulatory openings are present in the epigyne. Instead the epigyne is characterized by longitudinal folds, which extend deeply and are connected to the internal part of the genitalia. According to Kraus & Kraus (1988), the vulva consists of three intergrading parts (anterior tips, an intermediate region, and posterior multilocular receptacular cavities). The anterior tips are located in front of the copulatory opening (fold) and are equipped with glandular structures (Kraus & Kraus 1988). Interestingly, this part is somewhat ambiguously defined. For example, Miller et al. (2012) named it ''spermathecal head'' whereas Řezáč et al. (2008) defined it as ''copulatory ducts''. Certainly, this different terminology also implies different functions as copulatory ducts are used for the transport of sperm into the spermatheca whereas the spermathecal head as part of the spermatheca is likely related to sperm storage. In contrast, the spermathecae in the abovementioned studies resembles the receptacular cavities described by Kraus & Kraus (1988). However, it is not known where sperm storage takes place. Thus, we addressed following questions in this study: Where is sperm stored? What kind of epithelial tissue is associated with the sperm storage organ? How does that tissue differ from the epithelium associated with spermathecae? In order to answer these questions we investigated the female genital tract of *Eresus kollari* Rossi 1846 by means of light and electron microscopy.

METHODS

Eresus kollari occurs mainly in rocky steppes of the alliance *Festucion valesiacae* in Central Europe (Řezáč et al. 2008). Spiders of this species spend most of their lives underground in

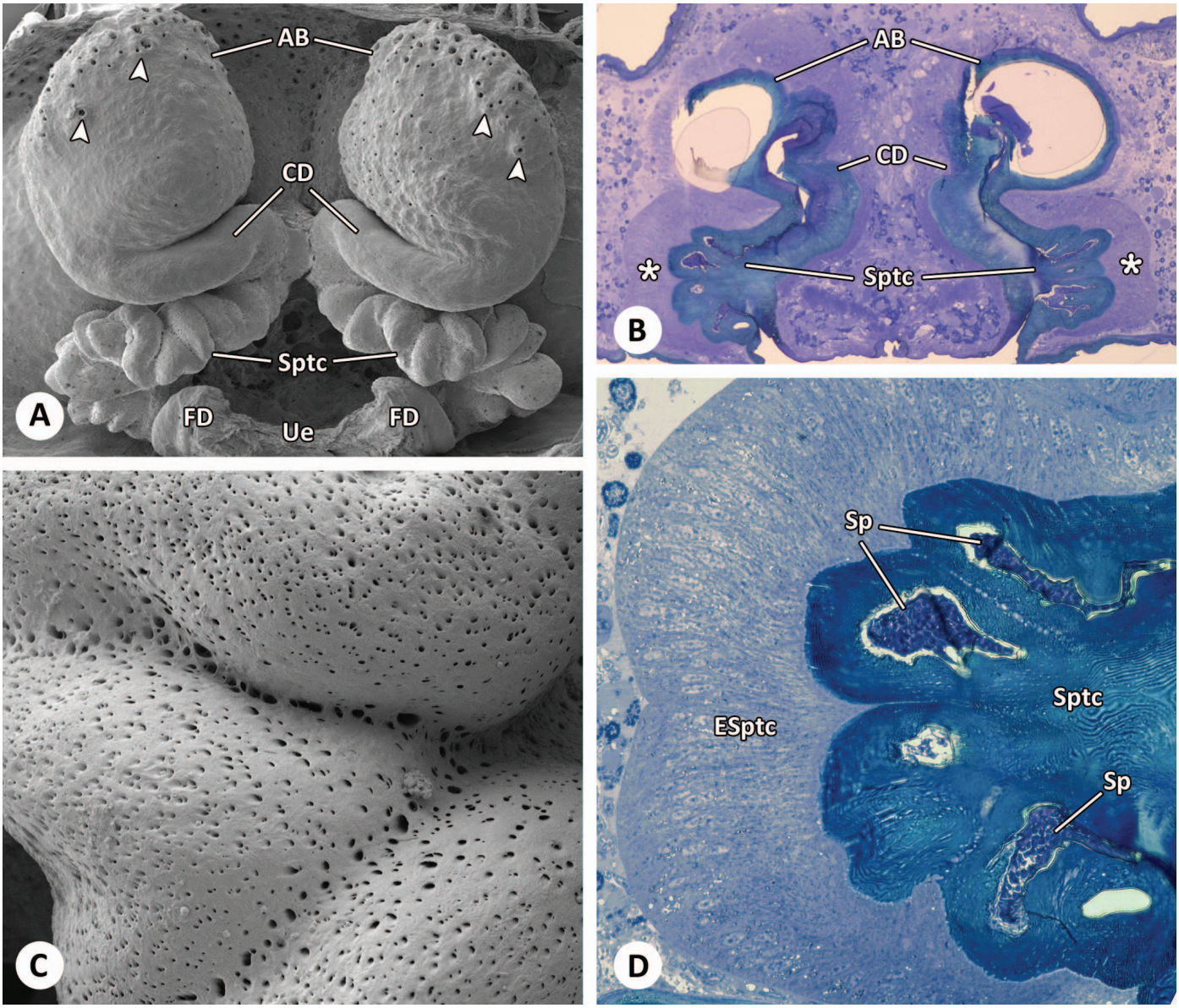

Figure 1A–D.—Overview of the vulva. SEM and LM. A. Dorsal view of the vulva; B. Transverse section of the vulva (asterisks mark the spermathecal epithelium); C. Detail of the cuticle of the spermatheca; D. Section of the spermatheca. Abbreviations: AB, anterior bulge; CD, copulatory duct; ESptc, epithelium of spermatheca; FD, fertilization duct; Sp, spermatozoa; Sptc, spermatheca; Ue, uterus externus.

well-camouflaged tube webs (Baumann 1997); their most common prey is beetles (Bellmann 1992; Kofler & Mildner 1993; Leist 1994; Baumann 1997; Walter 1999). We collected adult females after mating (indicated by the presence of a mating plug) from their burrows at three different localities in North Bohemia (Czech Republic) during September 2013: Ctiněves, Říp hill (50°23'2.986"N, 14°17'22.811"E); Obrnice, Zlatník hill (50°30'52.424"N, 13°42'54.487"E); Klapý, Hazmburk hill (50°25'59.093"N, 14°0'57.017"E). Voucher specimens are deposited in the Crop Research Institute, Prague (Czech Republic).

For scanning electron microscopy, the genitalia of five females were dissected and soft tissue was digested using Pancreatin (Álvarez-Padilla & Hormiga 2008). Specimens were dehydrated in graded acetone series, critical-point dried, sputter-coated with gold and examined in the JEOL JSM-7401F.

For light and transmission electron microscopy, genitalia of five females were dissected in a solution of 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4). Samples were post-fixed in osmium tetroxide for 2h, washed for another 1h and dehydrated in a graded series of acetone and then embedded in Spurrs resin (Spurr 1969). Semi-thin sections (1 μm) were cut with glass knives using a Leica UCT microtome, stained with toluidin blue and examined under a light microscope (Olympus CX41 with Olympus E510 IS digital camera). Ultrathin sections (80–160 nm) were cut with both glass knives and a diamond knife, stained with uranyle acetate, counter-

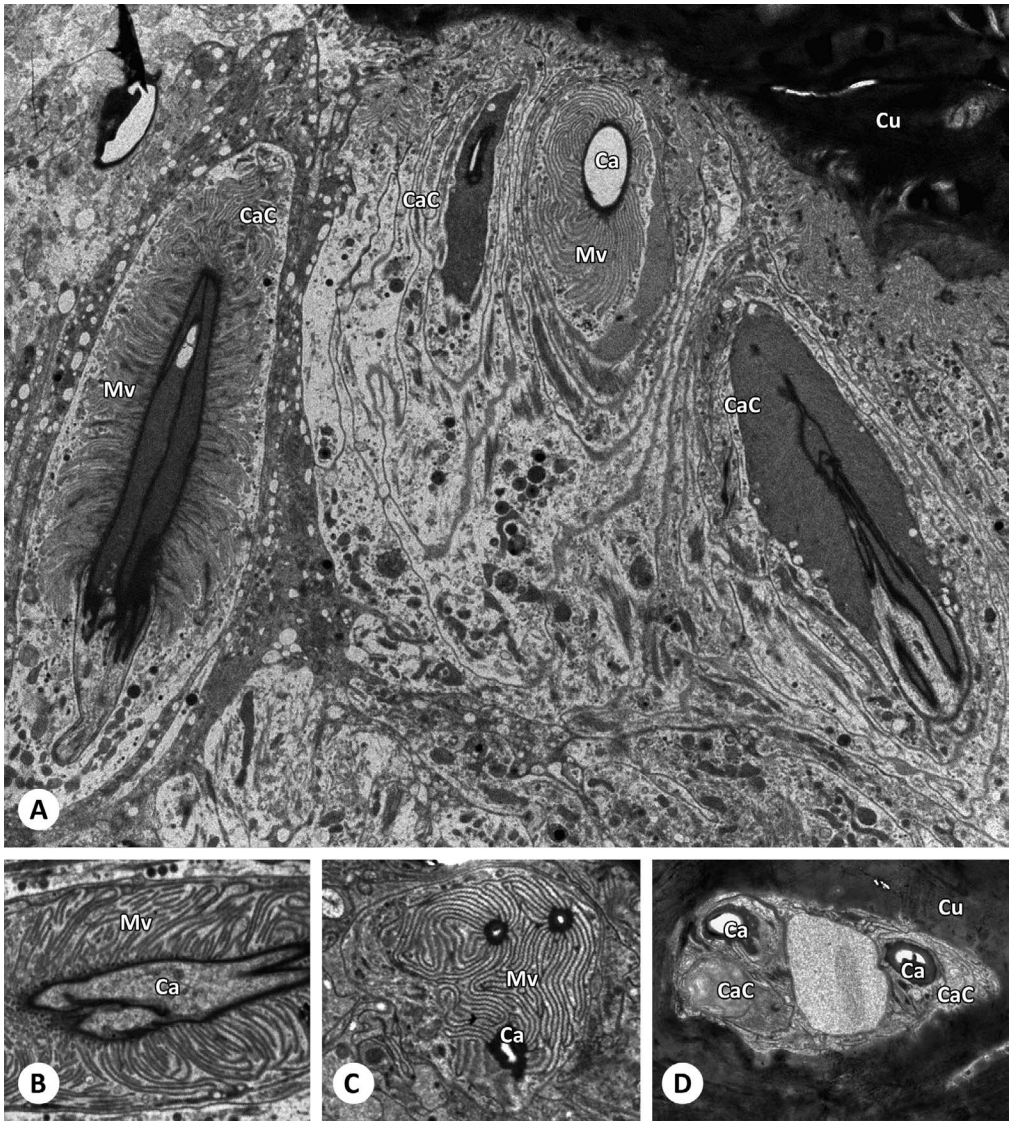

Figure 2A–D.—Glandular epithelium of the anterior bulge. TEM. A. Overview of the region close to the cuticle showing several canal cells. B, C. Longitudinal and cross-section of the cuticular ducts in the canal cell. D. Cross section through a cuticular pore showing two canal cells with cuticular ducts. Abbreviations: Ca, cuticular duct; CaC, canal cell; Cu, cuticle; Mv, microvilli.

stained with lead citrate (Reynolds 1963) and examined using a JEOL JEM-1010 electron microscope.

RESULTS

The epigyne of *E. kollari* is depicted in Řezáč et al. (2008) and is characterized by an anterior wide longitudinal bar and folds which are incurvated sidewards. The anterior end of these folds corresponds to the enlarged anterior bulge that is connected to a distinct copulatory duct (Fig. 1A). The copulatory opening is fold-like and situated posterior to the anterior bulge. The anterior bulge is equipped with numerous large pores (up to 10 μm) that are connected to a thick layer of the glandular epithelium (Figs. 1A & B, 2). The epithelium is composed of highly elongated gland units consisting of sheath, canal and secretory cells (Fig. 2). Most conspicuous are the canal cells, which are characterized by extensive microvilli and a thin-walled complex duct (Fig. 2A–C). The canal and secretory cells are surrounded by highly interdigitated sheath cells (Fig. 2A). The large pores may contain one or two cuticular ducts (Fig. 2D) that likely discharge glandular products into the bulge lumen. Numerous mitochondria and small vesicles are found in all cell types (Fig. 2). Secretory cells contain the largest number of small vesicles and a considerable number of large vesicles. Sheath cells are characterized by a large number of microtubules and bright cytoplasm, and they carry no microvilli.

The spermathecae consist of lobes that vary in number, shape and size (Fig. 1). The cuticle of the spermathecae is perforated by a large number of small pores (0.1–3 μm). These pores connect to a conspicuous epithelium that is most developed in areas around the lobes and reaches a thickness of approximately 70 μm. We could only detect one type of cell in the spermathecal epithelium, which is characterized by an extensively folded basal plasma membrane resulting in

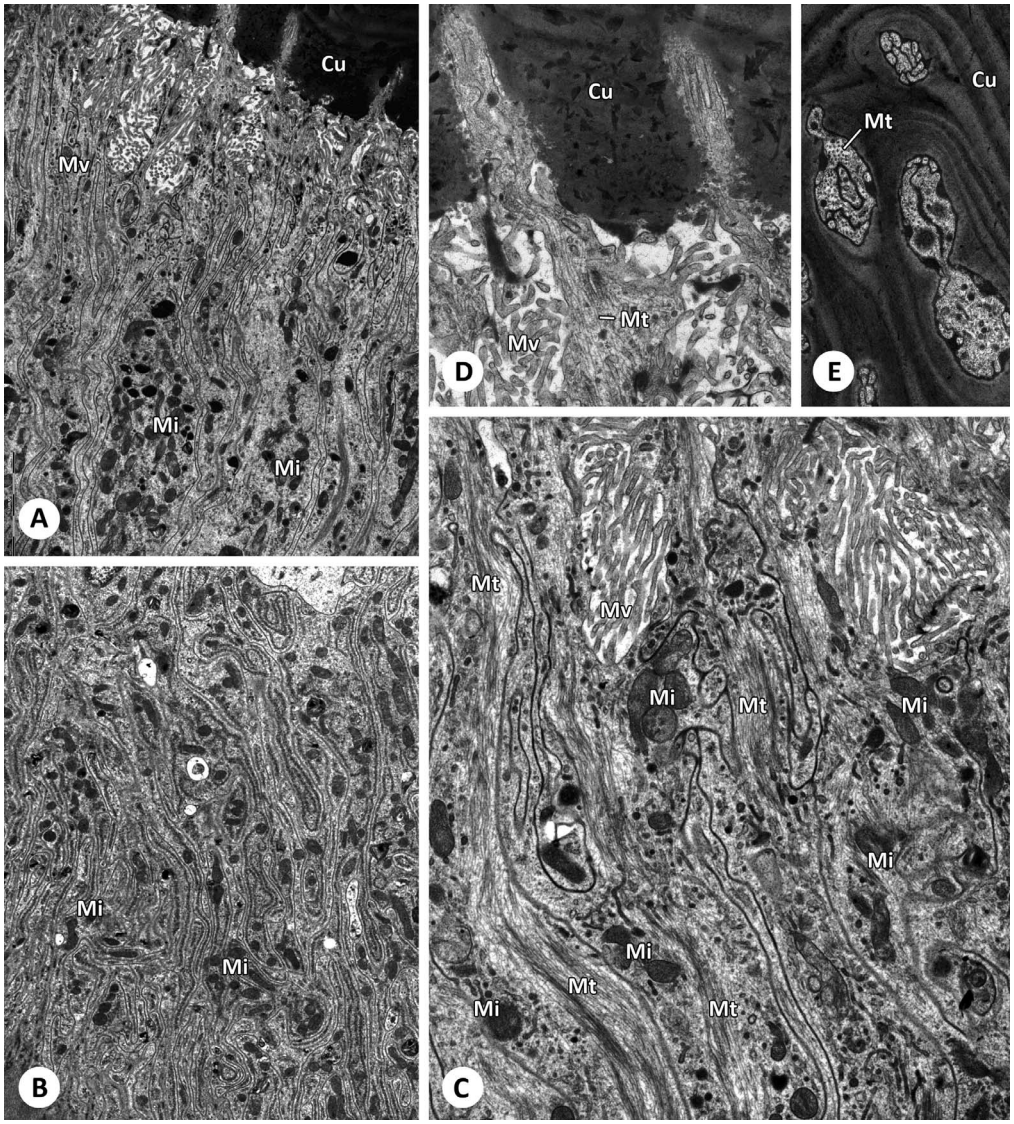

Figure 3A–E.—Epithelium of spermathecae. TEM. A, B. Apical and basal region of the epithelium. The basal labyrinth is very prominent and mitochondria are present in high quantities. C, D. Apical part of the cell which has an invaginated apex and bears numerous microvilli. The cells are connected by desmosomes and contain numerous mitochondria and bundles of microtubules. E. Cross-section through the cuticular pores showing the extension of the epithelial cells, which are characterized by numerous microtubules. Abbreviations: Cu, cuticle; Mi, mitochondria; Mt, microtubules; Mv, microvilli.

a prominent basal labyrinth (Fig. 3A, B). The basal labyrinth extends more than two third of the cells height. The nucleus and some endoplasmic reticulum are located in the basal part of the cell. The cell apex is invaginated and bears numerous microvilli surrounding extracellular space connecting to the spermathecal lumen through the pores (Fig. 3A, C, D). Thin extensions of the spermathecal cells extend into the pores and are characterized by numerous microtubules (Fig. 3E). The cells contain a high number of mitochondria, which can be oval-shaped or elongated (Fig. 3A–C). Moreover, small vesicles and bundles of microtubules are present (Fig. 3C).

In all studied specimens, sperm was only present in the spermathecae, not in the anterior bulge. The spermatozoa were encapsulated and densely packed within the lobes of the spermathecae (Fig. 1D).

DISCUSSION

Eresus kollari genetalia resemble the typical organization of eresid female genitalia with an epigyne characterized by two conspicous folds leading into a tripartite vulva (Kraus & Kraus 1988). As sperm was always absent in the anterior bulge and only present in the lobulated spermathecae, we conclude that the bulge is not involved in sperm storage. Thus, the term ''spermathecal head'' used by Miller et al. (2012) is misleading as it implies some role in sperm storage. Whether the anterior bulge is somehow involved in the mating process cannot be answered based on our data. However, as already described by Kraus & Kraus (1988), the copulatory opening is located posteriorly to the anterior bulge and thus the bulge might not be involved in anchoring of the male genitalia. Other potential

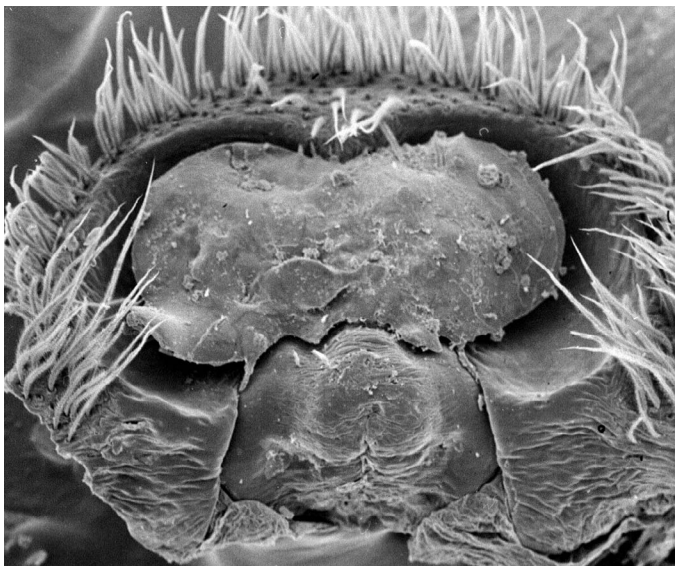

Figure 4.—Ventral view of the epigyne after copulation showing the amorphous mass which covers large parts of the epigyne including the copulatory pores. Usually, the plug occurs only in the anterior part of longitudinal folds. SEM.

functions may be explained by the presence of the glandular epithelium around the anterior part of the bulge. We found numerous glandular units that resemble the class 3 gland organization according to the classification of Noirot & Quennedey (1974, 1991). This type of glandular unit is typically found adjacent to sperm storage organs as it likely contributes products for the nutrition of spermatozoa (e.g., Uhl 1994a,b; Suhm & Alberti 1996; Uhl 2000; Berendonck & Greven 2005; Michalik et al. 2005; Useta et al. 2007). For example, Coyle et al. (1983) reported carbohydrates and glycogen for the spermathecal gland cells of some species of *Antrodiaetus* Ausserer 1871. Because no spermatozoa are stored in the bulge, we hypothesize that the bulge secretion could be involved in the transport of sperm by flushing a considerable quantity of secretion towards the posterior. Another possible function might be related to the amorphous mass that appears on the epigyne after copulation (Fig. 4). This amorphous mass consists of secretion and may resemble a mating plug as described for many different spider taxa (Uhl et al. 2010). Interestingly, a mating plug was also reported for several species of another eresid genus, *Stegodyphus* (for example, Figs. 26 and 259 in Kraus & Kraus 1988). However, whether this mass really prevents consecutive matings must be addressed in future behavioral and genetic studies. Although males usually produce such plugs, a female contribution to the production of such mass could be possible and has been reported for several other entelegyne taxa (e.g., Knoflach 1998, 2004). Thus, future studies on eresid genitalia should consider a role of the anterior bulge in the production of the amorphous mass as a potential prevention of female remating.

Another peculiar feature of the female genital system of *E. kollari* is the spermathecal epithelium. As mentioned above, usually class 3 glands are present around the sperm storage organs of female spiders. Alternatively, Uhl (2000) reported another gland cell type for the female genitalia of the dysderid *Dysdera erythrina* (Walckenaer 1802). In addition to the class 3 cells that occur on the anterior spermatheca, she reported class 1 gland cells to be present on the large posterior diverticulum. The occurrence of two different types of glands likely results in different sperm storage condition and is thus highly relevant to addressing the function of the different storage sites (Uhl 2000). However, the spermathecal epithelium of *E. kollari* does not consist of typical gland cells because only a few secretory droplets and endoplasmic reticulum are present. Instead, the cells are characterized by a very prominent basal labyrinth and numerous partly elongated mitochondria. Moreover, the cell apex is invaginated and bears numerous microvilli forming an apical complex. These features resemble the organization of transport epithelia, which are, for example, found in coxal and other complex glands (e.g., Rosenberg 1983; Alberti & Coons 1999; Rosenberg et al. 2006). The main function of transport epithelia is the transport of ions that may lead in the case of *E. kollari* to an alteration of the storage condition in the spermathecae. However, it is not clear how such change in the milieu in the lumen of the spermatheca affects the spermatozoa. Females of *E. kollari* store sperm for months, as mating occurs in early autumn but oviposition does not occur before spring. Because we only studied females during the mating season, we do not know when sperm cells become active and whether spermatozoa need nutrients from the female to stay viable given that they often contain glycogen and mitochondria (Michalik & Ramírez 2014). Sperm of *E. kollari* are encapsulated and very densely packed in the spermathecae and we could not detect extensive amounts of secretion. Moreover, the timing of sperm activation varies depending on the species as shown for variety of spider taxa (Eberhard & Huber 1998; Useta et al. 2007; Vöcking et al. 2013) and thus a general inference cannot be made given the present state of knowledge.

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