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FINE STRUCTURE OF THE MALE GENITAL SYSTEMS, SPERMATOPHORES AND UNUSUAL SPERM CELLS OF SAXIDROMIDAE (ACARI, ACTINOTRICHIDA)

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ABSTRACT — The early derivative actinedid Saxidromidae, *Saxidromus delamarei*, *Bovidromus roussouwi*, and *Rhinodromus lootsi* perform indirect spermatophore transfer by means of a peculiar mating behaviour. The anatomy and fine structure of the male genital systems are described and are shown to exhibit the organisation considered to be fundamental in Actinotrichida: paired testes with germinal and glandular parts, paired vasa deferentia, unpaired ejaculatory duct, progenital chamber containing genital papillae. The genital system is located ventral of the digestive tract. The testes produce relatively few or even very few aflagellate sperm cells, which represent synspermia consisting of likely four undivided sperm cell equivalents in a common cytoplasm. Whereas *S. delamarei* produces a rather large stalked spermatophore containing many synspermia, both the other species produce very tiny stalked spermatophores with a spherical head representing only one synspermium. Thus, in these latter species only one synspermium is transferred to the female at a time, but the male can convey several (possibly five or more) spermatophores to the female during the mating session. A scenario is suggested which could describe the evolution of this remarkable mating system and behaviour.

KEYWORDS — evolution; mating behaviour; mating economy; sperm priority; synspermia; ultrastructure

INTRODUCTION

Saxidromidae comprising three genera, *Saxidromus*, *Bovidromus* and *Rhinodromus*, is a family of mites regarded to be close to the basis of the species-rich and highly diverse Prostigmata (Coineau, 1979; Coineau *et al.*, 2006). However, some authors include the few species known into the Adamystidae, which is considered to represent an early derivative group within the very diverse Anystides (Evans, 1992; Krantz and Walter, 2009). All species known occur on bare rocks, which are exposed to bright sunlight. They live in rock crevices but appear on rock surfaces from early summer to mate. Despite the different systematic views on their rather basal position, they are known to perform a very unusual mating behaviour (Coineau, 1976; Coineau *et al.*, 2006).

The male catches a female with its forelegs and impales it (*S. delamarei*) onto or place it over a sper-

matophore previously produced. Males of *B. roussouwi* provided with two and *R. lootsi* possessing one dorsoanteriad projecting processus of the idiosoma insert these into the female's progenital chamber prior to spermatophore deposition. In contrast to *S. delamarei*, which deposits a rather large stalked spermatophore, these latter two species deposit very tiny spermatophores bearing a small spherical droplet. The female takes only the droplet and the stalk can be replenished several times by the male with a further droplet, which contains sperm material. Following a first investigation on *Saxidromus delamarei* (Alberti *et al.*, 2007), we have studied the fine structure of the male genital systems, the spermatophores and sperm of two further species and found remarkable and peculiar similarities but also pronounced differences.

MATERIALS AND METHODS

Specimens of *Saxidromus delamarei* Coineau, 1974 were collected in June 2003 by Y.C. and N.A.F. close to Banyuls-sur-Mer (France), whereas specimens of *Bovidromus roussouwi* Coineau, Théron and Fernandez, 2006 were sampled near Potchefstroom (South Africa) and *Rhinodromus lootsi* Coineau, Théron and Fernandez, 2006 near Rustenburg (South Africa) by P.Th and G.A. in March 2005.

Transmission electron microscopy (TEM) *and light microscopy* (LM): The living mites were transferred into a drop of cold fixative (3.5% buffered glutaraldehyde; phosphate or cacodylate buffer 0.1M; pH 7.4) and transversely cut into halves. The specimens were immediately transferred into small vials containing the fixative and placed into a refrigerator for about two hours. They were then rinsed several times with the buffer solution and transferred for postfixation into 2% buffered OsO_4 solution, where they were left for another two hours. The South African specimens were transferred into diluted glutaraldehyde (1:4 with buffer solution) and were further processed after return of G.A. to Greifswald. Following the OsO_4-fixation the specimens were rinsed several times with buffer solution and finally dehydrated using graded ethanols. Embedding occurred either in Araldite with propylenoxide as intermedium or in Spurr's mixture (Spurr 1969). The living mites were found to be very hydrophobic, hence the process of fixation was slightly varied to improve the results when working with the South African species. These mites were briefly immersed into isopropanol to break the hydrophobic barrier and thus to facilitate the access of the fixatives. These problems did not occur with the spermatophores of *S. delamarei*, which were collected immediately after deposition and treated as described. Because of the tiny size of the spermatophores of the South African species, it was not possible to collect individual spermatophores. However, we were able to obtain mating mites during spermatophore transfer and hence got the heads of the spermatophores. They were fixed together with the mites.

Sectioning was done with a Leica ultracut UCT microtome, whereby ultrathin sections were produced (70 nm) - and from time to time semithin sections (400 nm) - using a Diatome diamond knife. The ultrathin sections were stained with uranylacetate and lead citrate according to Reynolds (1963) and studied in a Zeiss EM10A or (mostly) JEOL-JEM-1011 (80 kV) transmission electron microscope. The semithin sections after staining according to Richardson *et al.*, (1960) served for general orientation using a light microscope (for further technical details see, e.g., Alberti and Nuzzaci, 1996). Micrographs of these semithin sections were taken with an Olympus BX60 microscope provided with an Axio Cam MRC - Zeiss digital-camera connected with the Axio Vision Rel. 4.8 program.

RESULTS

Male genital system

The male genital system of all three species consists of two tubular testes located in the posterior of the body which continue anteriad into a pair of vasa deferentia extending beyond the genital opening (Figure 1a). A transversal bridge shortly in front of the genital opening connects both of the deferent ducts (Figure 1b). This unpaired structure opens posterioventrad into the cuticle-lined dorsal chamber of the ejaculatory duct, which descends almost

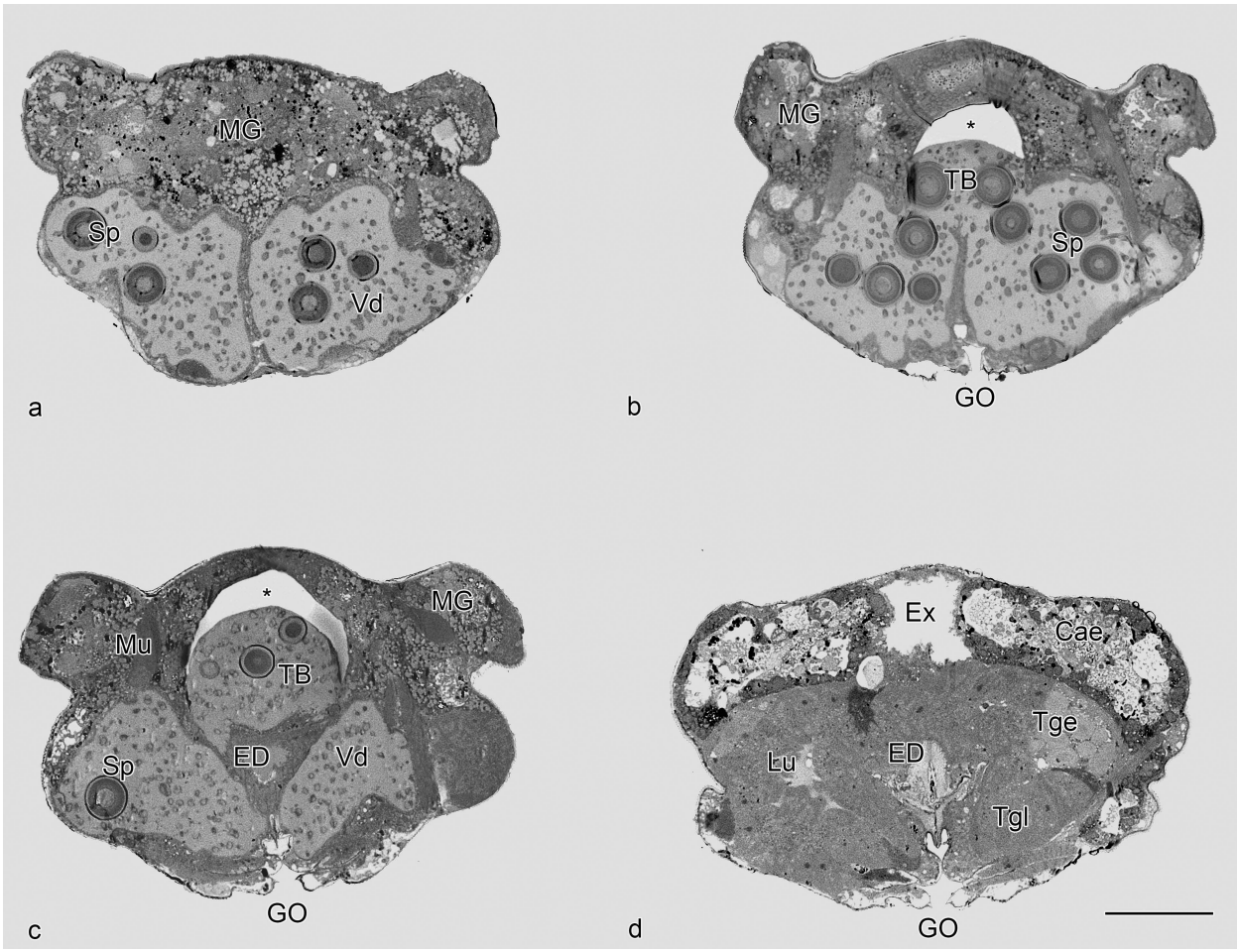


FIGURE 1: Light micrographs of transverse sections through the posterior part of the idiosoma of *Rhinodromus lootsi*. (a) – Shortly in front of the genital opening. The two vasa deferentia located below the midgut are seen containing several synspermia. (b) – Slightly posterior, the genital opening is appearing. The vasa deferentia are connected by a transverse bridge (asterisk indicates a shrinkage artifact). (c) – More posteriorly, the ejaculatory duct with its dorsal chamber appears (asterisk indicates artifact). (d) – The paired testis are seen consisting of germinal and glandular parts. The dorsomedian excretory organ (i.e. the postcolon; e.g., Alberti and Coons 1999) is seen. Note that all parts of the genital system are located ventral of the digestive system. Scale bar: 50 μm.
Abbr.: Cae, caecum of midgut; ED, ejaculatory duct; Ex, excretory organ; GO, genital opening; Lu, lumen of testis; MG, midgut; Mu, dorsoventral muscles; Sp, synspermia; TB, transversal bridge; Tge, germinal part of testis; Tgl, glandular part of testis; VD, vas deferens.

vertically (Figures 1c,d and 3). The primary or eugenital opening is located within a progenital chamber, which includes aside of eugenital setae two pairs of genital papillae and is closed by progenital lips (terms acc. to van der Hammen, 1980). The genital system is located ventral of the digestive tract (Figure 1a-d).

The testes consist of two parts both extending longitudinally through the structure, a small germinal part and a prominent glandular part. Within the germinal part only few or even very few germ cells were seen (Figures 1d and 2a). Because of the rarity of germ cells, spermatogenesis could not yet be studied in detail. In any case, these cells are rather large and do not separate at the end of spermiogenesis into individual cells (spermatozoa).

Hence aggregates containing (most likely in all three species) four sperm equivalents in a common cytoplasm are delivered into the lumen of the testis. These structures represent so-called synspermia.

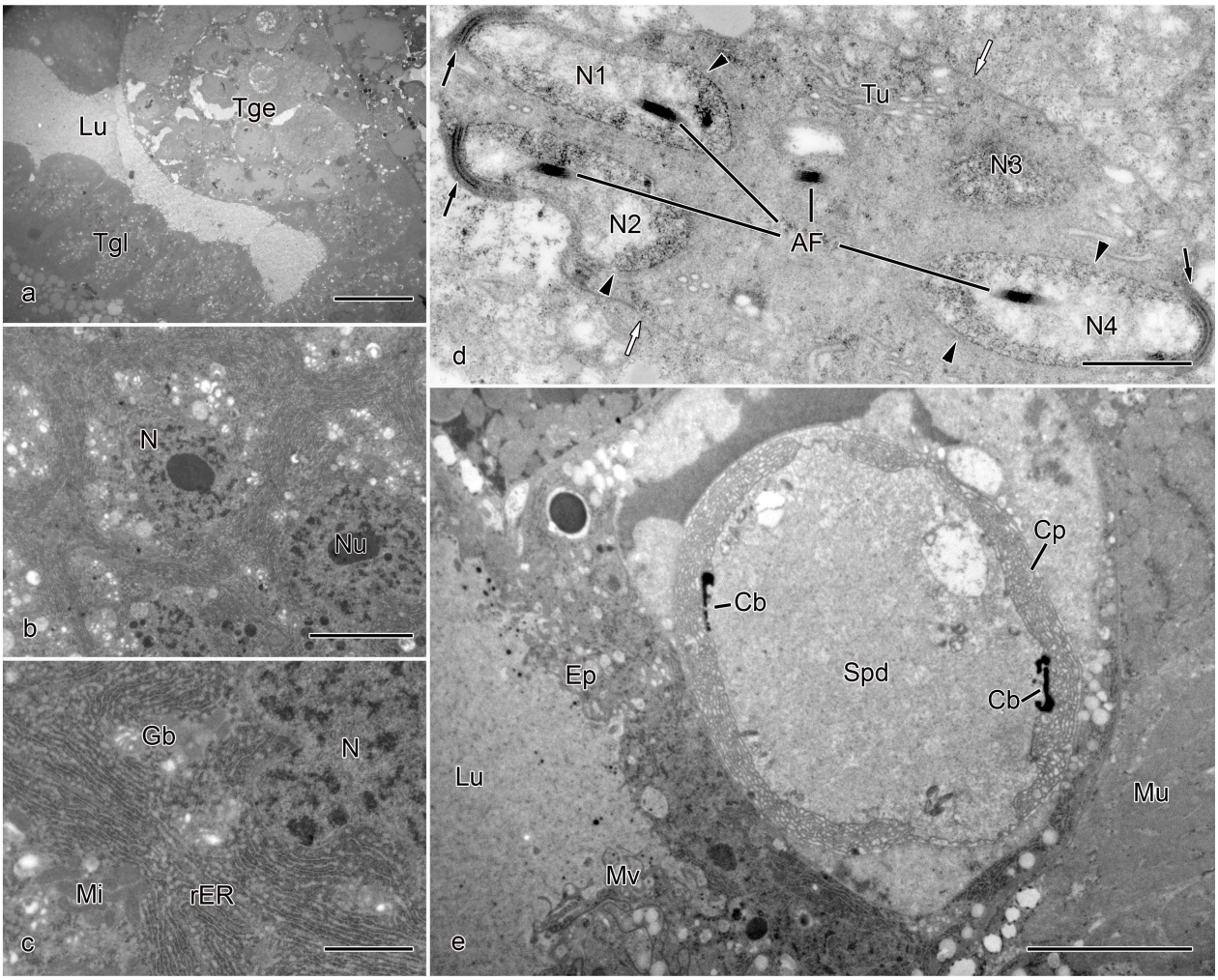


FIGURE 2: TEM micrographs of details of testis of *Rhinodromus lootsi* (a-c, e) and *Saxidromus delamarei* (d). (a) – Overview of testis. Note thick epithelium comprising the glandular part and area with round spermatids composing the germinal part of testis. Scale bar: 20 μm. (b)– Detail of glandular part showing large nuclei, conspicuous nucleoli and Golgi bodies. Scale bar: 5 μm. (c) – Nuclear region of glandular epithelial cell with numerous rough ER cisternae and Golgi bodies. Scale bar: 2 μm. (d) – Detail of germinal part of testis of *S. delamarei* showing synspermatid containing four nuclei (N1-N4). White arrows indicate cell membrane bordering the synspermatid. Note tubular invaginations at the cell peripheriy. Three acrosomal vacuoles (black arrows) and parts of acrosomal filaments are also seen. The nuclei, at the beginning of chromatin condensation, still have a nuclear envelope (arrowheads). Scale bar: 1 μm. (e) – Distal part of testis with large synspermatid. Two much condensed chromatin bodies are seen. Scale bar: 5 μm.
Abbr.: AF, acrosomal filament; Cb, chromatin body; Cp, cell protrusions; Ep, epithelial cell; Gb, Golgi body; Lu, lumen; Mi, mitochondria; Mu, muscle; N, nucleus; Nu, nucleolus; rER, rough endoplasmic reticulum; SPd, synspermatid; Tge, germinal part of testis; Tgl, glandular part of testis; Tu, tubular invaginations.

The glandular part consists of large epithelial cells, which are provided with large nuclei containing a prominent nucleolus. The cytoplasm is dominated by rough endoplasmic reticulum and small electron-lucent Golgi bodies (dictyosomes) (Figures 1d and 2a,c).

A secretion is produced by these cells and fills the lumen of the testes, vasa deferentia and unpaired chamber of the ejaculatory duct together with the mature sperm cells (Figure 1).

The secretion consists of several components. In, e.g., *Saxidromus delamarei* it consists of large electron-dense droplets and a matrix of peculiar, probably fibrous, substructure (Figure 5a). It con-

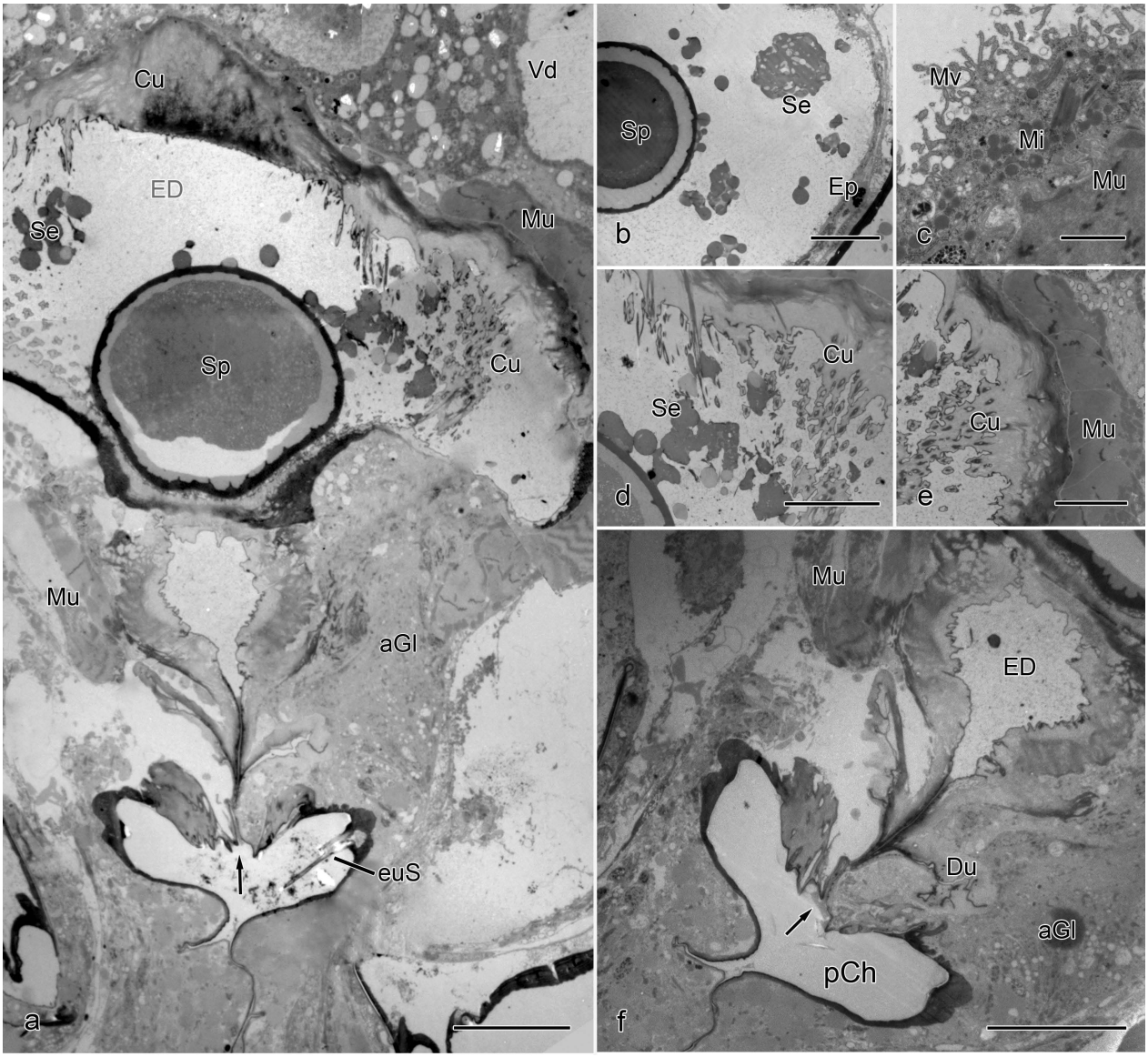


FIGURE 3: TEM micrographs of vas deferens and ejaculatory duct of *Bovidromus roussouwi*. (a) – Composed figure showing complex structure of ejaculatory duct in cross section. Note large synspermium in dorsal chamber lined by peculiar cuticle. Arrow points to eugenital (primary) genital opening. Scale bar: 20 μm. (b) – Vas deferens. Note flat epithelium. Scale bar: 20 μm. (c) – Detail of epithelium of vas deferens with irregularly shaped microvilli. Cells contain many mitochondria and are underlain by a muscular layer. Scale bar: 2 μm. (d) – Dorsal chamber of ejaculatory duct with cuticular fringes and secretion. Scale bar: 10 μm. (e) – The ejaculatory duct is surrounded by a thick muscular layer. Scale bar: 10 μm. (f) – Eugenital opening (arrow) and accessory gland. Scale bar: 20 μm.
Abbr.: aGL, accessory gland; Cu, cuticle; Du, duct of accessory gland; ED, ejaculatory duct; Ep, epithelium; euS, eugenital seta; Mi, mitochondria; Mu, muscle; Mv, microvilli; pCh, progenital chamber; Se, secretion; Sp, synspermium; Vd, vas deferens.

tributes to the formation of a secretion layer around each synspermium (Figure 3a,b).

Due to technical problems with the fixation (see Materials and methods), the distal parts of the genital system were best observed in the South African species and the following description of these parts are largely based on these species (Figures 1, 3 and 4).

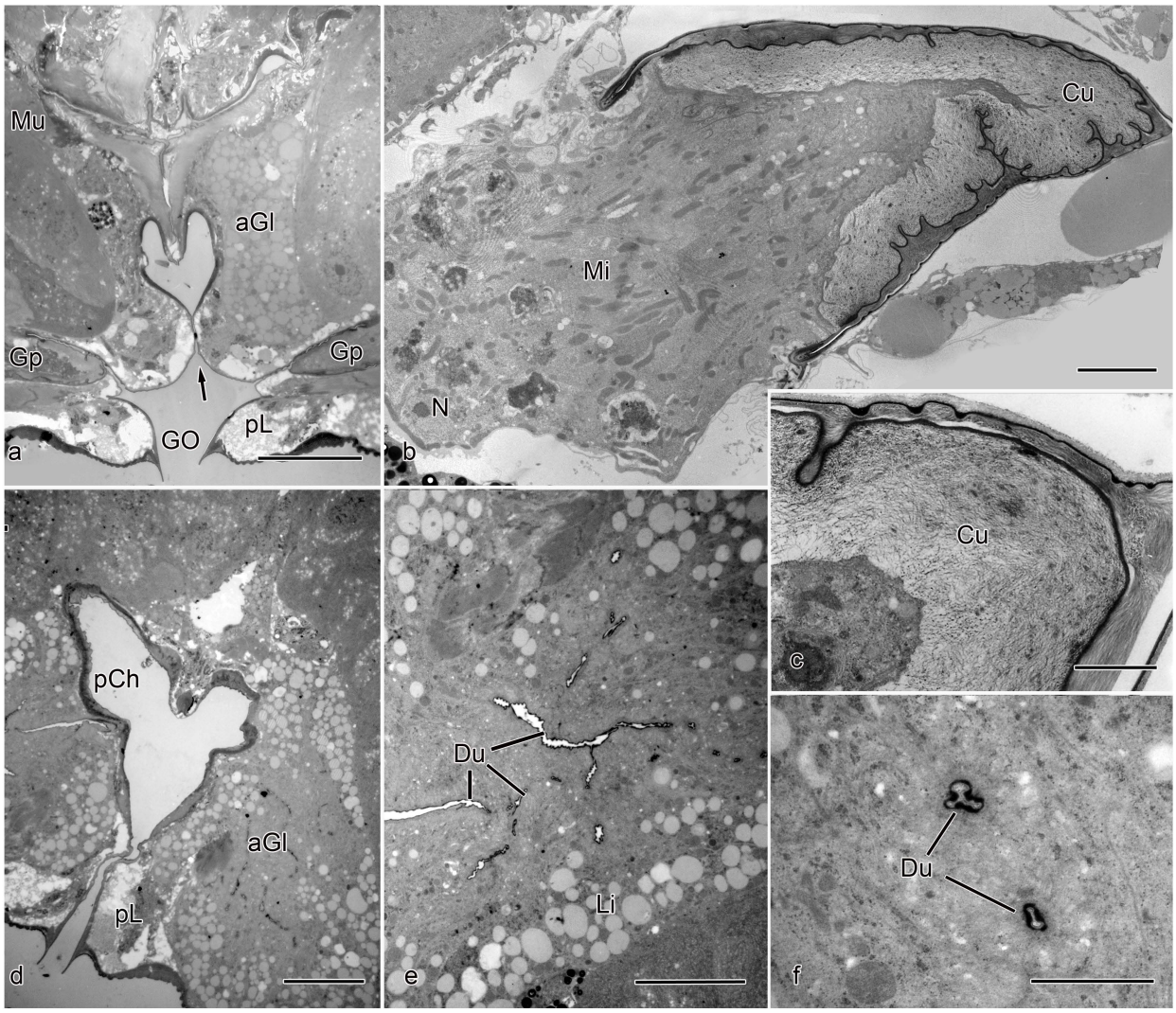


FIGURE 4: : TEM micrographs of transversely sectioned progenital chambers of *Rhinodromus lootsi* (a, d-f) and *Bovidromus roussouwi* (b, c). (a) – Posterior part of ejaculatory duct with eugenital opening (arrow) and secondary genital opening bordered by progenital lips. Note accessory gland and position of (retracted) genital papillae. Scale bar: 20 μm. (b) – Genital papilla. Note many mitochondria and peculiarly modified cuticle. Scale bar: 5 μm. (c) – Detail showing modified cuticle of genital papilla. Note the thick cuticle consisting mainly of many loosely arranged fibres and thus giving a cushion-like appearance. Scale bar: 1.2 μm. (d) – Progenital chamber and accessory glands. Scale bar: 20 μm. (e) – Accessory gland with branching ducts. The cells contain numerous lipid inclusions. Scale bar: 10 μm. (f) – Detail showing two sections through thin ducts, which are cuticle-lined. Scale bar: 2 μm.
Abbr.: aGl, accessory gland; Cu, cuticle; Du, duct of accessory gland; GO, secondary genital opening; Gp, genital papilla; Li, lipid inclusions; Mi, mitochondria; Mu, muscle; N, nucleus; pCh, progenital chamber; pL, progenital lip.

The vasa deferentia have a wide lumen which is surrounded by a flat epithelium. The epithelial cells bear irregularly shaped microvilli. Rather many mitochondria are obvious in these cells, which are underlain by a muscular layer. The transversal bridge collects the mature sperm prior to transfer and delivers them into the dorsal chamber of the ejaculatory duct (Figure 3). The epithelium of this duct is cuticle-lined. The cuticle shows numerous spines or fringes which reach into the lumen of the duct. It is differently sclerotized according to the region within the duct. The shape of the ejaculatory duct is rather complex and could not yet be reconstructed completely. However, towards the genital opening

a funnel-like, narrow passage, is formed which terminates at the eugenital opening. Also this part of the duct is provided with a very complex arrangement of cuticular structures. The ejaculatory duct is surrounded by a strong muscular layer.

The genital papillae protruding laterally into the progenital chamber are located in deep pouches between proximal folds bordering the eugenital opening and the inner wall of the progenital lips. The few epithelial cells comprising the genital papillae show membrane folds, many mitochondria and microtubules. The overlying cuticle is conspicuously thicker and "cushion-like" when compared to the adjacent cuticle (Figure 4a-c).

A pair of accessory glands opens proximal to the eugenital opening into the ejaculatory duct. The cells of the accessory glands seem to be quite large and are full of electron-lucent lipid inclusions. A highly branching system of thin cuticle-lined ducts penetrates deeply into the gland (Figures 3a,f and 4d-f). Aside of the eugenital opening, eugenital setae are located (Figure 3a). The progenital lips border the (secondary) genital opening with sharp longitudinal cuticular keels (Figure 3a,d).

Spermiogenesis and sperm structure

The sperm cells of the three species are similar in so far as they represent synspermia as mentioned already (Figure 5). As in other spermatophore producing actinotrichid mites, the (syn)spermia are provided with a secretion layer (Alberti, 1980b; Alberti and Coons; 1999). Comparing the saxidromid synspermia, it is evident, that the sperm of the European species differ remarkably from the sperm of the two South African species being more similar to each other than to those of *S. delamarei*. The synspermia are aflagellate and spherical and show acrosomal complexes consisting of an acrosomal vacuole and an acrosomal filament (perforatorium), condensed nuclear material devoid of a nuclear envelope (so-called chromatin bodies), and a number of mitochondria. In each synspermium, several acrosomal complexes and chromatin bodies are present, likely four (indicating the spermatids which have not separated).

S. delamarei has the most complex sperm of the three species. Details have already been published elsewhere. Here only some characteristics are reported for comparison (Figure 5a-c; see Alberti *et al.*, 2007). The synspermium is rather small (diameter about 2.5 μm) and surrounded by a rather thin secretion layer (about 0.075 μm thick). Most conspicuous is the acrosomal complex which protrudes slightly against the secretion layer producing small bulges at the surface of the cell. The acrosomal vacuole of each sperm equivalent is highly structured showing a complex shape and including different components. It is underlain by a flat extension of the sausage-shaped chromatin body. The extension is penetrated by the acrosomal filament, which runs into the cytoplasm. Chromatin bodies and acrosomal filaments are sectioned many times because of their length and their meandering course within the cytoplasm (Figure 5a-c). The acrosomal filament is relatively thick (about 0.09 μm in diameter). Spermatids show many tubular invaginations of the plasmalemma (Figure 5b), which later are hardly visible in the mature sperm.

The sperm of *B. roussouwi* (diameter about 50 μm) and *Rh. lootsi* (diameter abbout 28 μm) are much larger due to an increased amount of cytoplasm and provided with a much thicker secretion layer (*B. roussouwi* about 4.6 μm, *Rh. lootsi* about 6.45 μm thick) (Figure 5d,g). In contrast to *S. delamarei*, the acrosomal complexes and chromatin bodies are rather small and inconspicuous in both species. They are also located at the cell periphery, but do not project. The acrosomal vacuoles are irregularly shaped, flat cisternae containing electron-dense material (Figure 5f,j). That of *R. lootsi* was rarely seen and my be very small (Figure 5h-j). They are underlain by numerous small filaments. Similar filaments were also seen adjacent to parts of the chromatin bodies. The acrosomal filament (in *B. roussouwi* about 0.19 μm, *Rh. lootsi* about 0.13 μm thick) starts more or less underneath a central invagination of the vacuole and runs into the cytoplasm after penetrating a similar irregularly shaped flat chromatin body. Since sections of the acrosomal filament were only seen close to the acrosomal complexes and chromatin bodies, the filament is likely

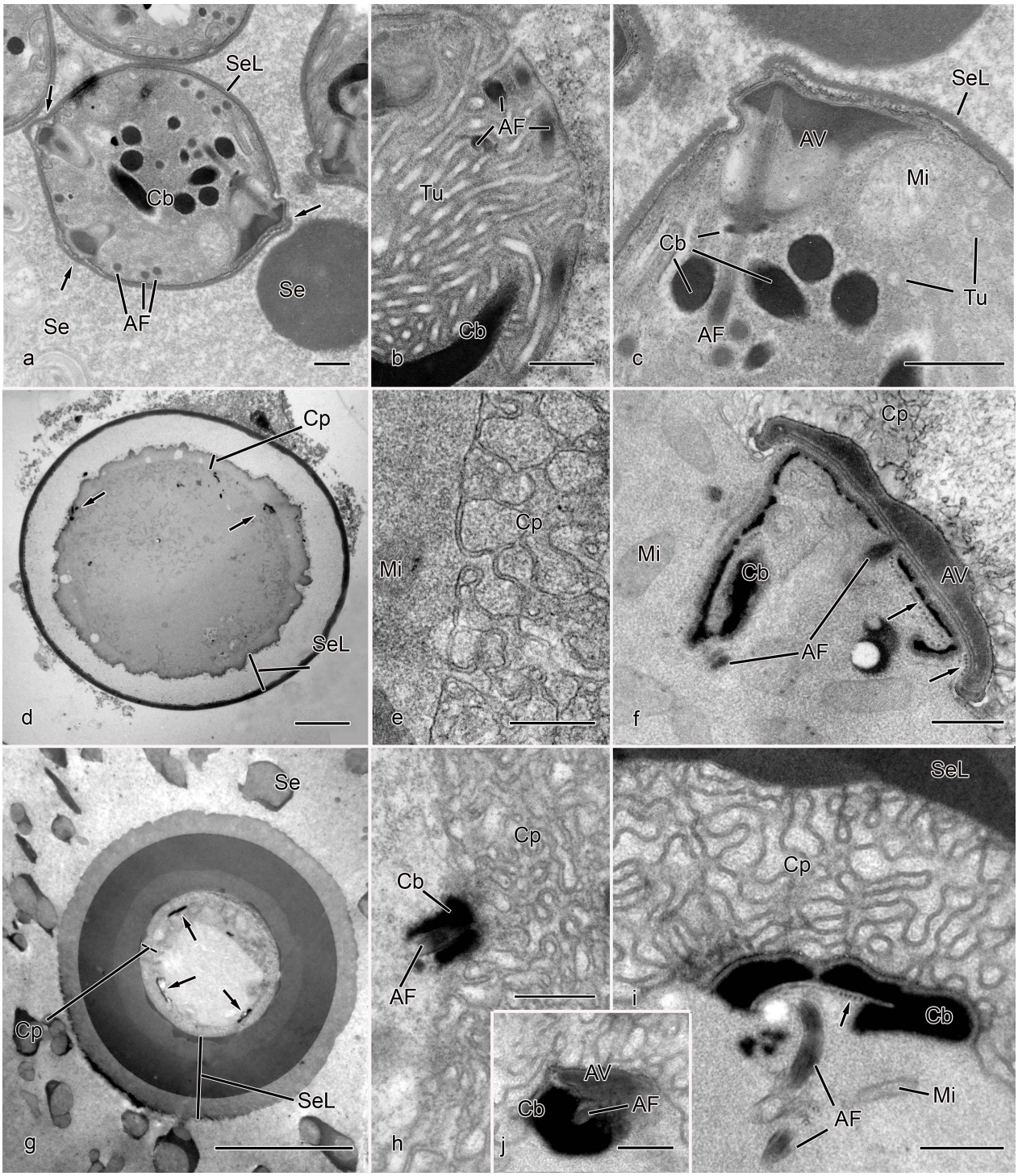


FIGURE 5: See next page

relatively short. A thick mantle of numerous mitochondria is located underneath the cell periphery. The centre of the synspermium seems to be largely devoid of organelles. In contrast to *S. delamarei*, the cell surfaces of the synspermia of both South African species are provided with numerous conspicuous interdigitating cell protrusions which are covered by the secretion layer mentioned above.

The layer is composed of several sublayers (most conspicuous in *Rh. lootsi*) as is obvious from Figure 5d and g.

Spermatophore structure

As with the synspermia, the spermatophores produced by the three species are more alike in the two South African species, whereas those of *S. delamarei* differ considerably (Figure 6). In this latter species, rather conventional stalked spermatophores (about 300 μm high) are deposited. They are rather simple, stout structures with a thick stalk and an elongated head which ends with a short attenuating tip. The sections reveal that the spermatophore is not much structured. There is an indistincly delimited chamber in the upper third of the spermatophore which contains a dense secretion and numerous synspermia (Figure 6b) and is surrounded by a considerable amount of additional electron-lucent secretion.

In contrast to *S. delamarei*, the spermatophores of the two South African species are smaller and much more slender (in *B. roussouwi* about 240 μm; *Rh. lootsi* about: 130 μm high; estimates according to drawings from Coineau *et al.*, 2006) consisting of a thin stalk and a spherical head. Hence we could only obtain the heads of the spermatophores (see Materials and methods). These heads were found to represent one synspermium as described above (Figures 1a-c, 3a,b and 5d,g).

DISCUSSION

The male genital system of Saxidromidae is fundamentally structured as in other actinotrichid mites. Most distinct is the organization of the testes into germinal and glandular parts and the location of the system ventral to the digestive tract (see, e.g., Alberti and Coons, 1999, Alberti, 2006). The ejaculatory duct opens into the progenital chamber which contains genital papillae, which are considered to play a role in water- and/or osmoregulation (Alberti, 1979; Alberti and Coons, 1999).

Except for the ejaculatory duct, which is highly structured, the entire system may be regarded as rather simple when compared to other actinedid mites, e.g., Bdellidae, Erythraeidae (Michael, 1896; Alberti, 1974; Witte, 1975). This may correlate with the rather simple structure of the spermatophores. Spermatophores occur in actinedid and oribatid mites and numerous have been described light microscopically (see, e.g., Alberti and Coons, 1999). In contrast, only few spermatophores have been studied with regard to fine structure, e.g., those of several Oribatida (e.g., Alberti *et al.*, 1991; Fernandez *et al.*, 1991). It turned out that these spermatophores,

FIGURE 5: TEM micrographs of spermatid/mature synspermia of *Saxidromus delamarei* (a-c), *Bovidromus roussouwi* (d-f), *Rhinodromus lootsi* (g-j). (a) – In *S. delamarei*, the sausage-shaped chromatin bodies are very obvious. Note three acrosomal complexes (arrows) projecting from the cell surface. The synspermium is covered by a thin secretion layer. Scale bar: 0.5 μm. (b) – A late synspermatid of *S. delamarei* showing the many tubular invaginations of the cell periphery. Scale bar: 0.5 μm. (c) – Acrosomal complex with peculiarly shaped acrosomal vacuole and acrosomal filament penetrating flat , plate-like extension of chromatin body. Scale bar: 0.5 μm. (d) – Synspermium of *B. roussouwi* with two inconspicuous chromatin bodies (arrows). Note thick secretion layer. Scale bar: 10 μm. (e) – Detail of cell periphery with interdigitating cell protrusions. Scale bar: 0.5 μm. (f) – Acrosomal complex showing a flat acrosomal vacuole underlain by a flat chromatin body. Note thin filaments (arrows) and acrosomal filament penetrating the chromatin body. Scale bar: 0.5 μm.(g) – Synspermium of *Rh. lootsi* showing very conspicuous secretion layer. Three small chromatin bodies are seen (arrows). Scale bar: 10 μm. (h) – Cell periphery with interdigitating cell protrusions and small chromatin body penetrated by acrosomal filament. Scale bar: 0.5 μm. (i) – Another aspect of cell periphery with chromatin body and acrosomal filament. Note thin filaments (arrow). Scale bar: 0.5 μm. (j) – A further aspect of acrosomal region with chromatin body covered by small acrosomal vacuole. Arrow points to thin filaments underneath chromatin body. Scale bar: 0.5 μm.
Abbr.: AF, acrosomal filament; AV, acrosomal vacuole; Cb, chromatin body; Cp, cell protrusions; Mi, mitochondria; Se, secretion of variable appearance filling genital tract lumen; SeL, secretion layer; Tu, tubular invaginations.

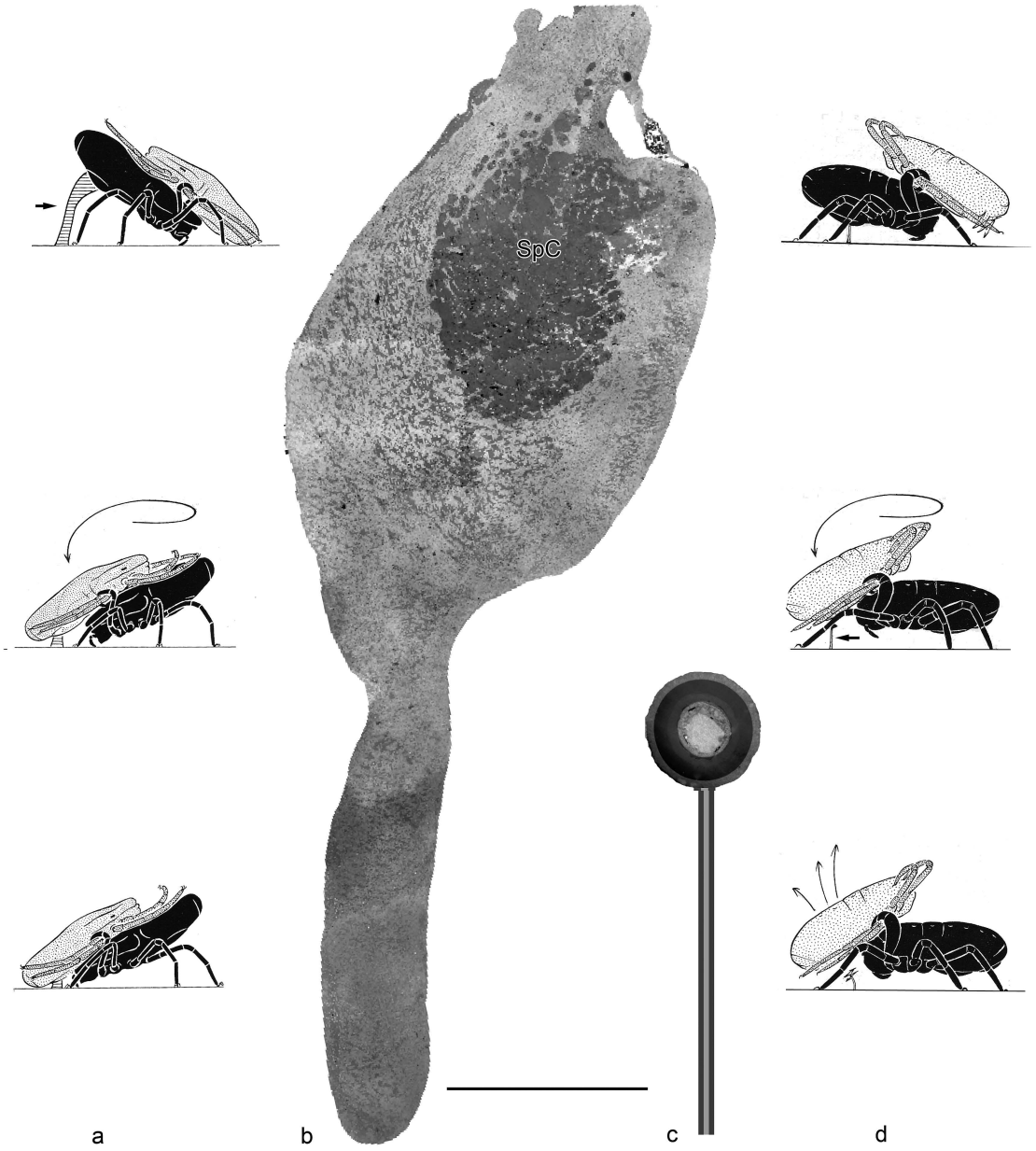


FIGURE 6: Comparison of mating by *Saxidromus delamarei* and *Rhinodromus lootsi* and their corresponding spermatophores as seen in TEM (that of *Rh. lootsi* partially reconstructed; a, d from Coineau *et al.*, 2006, b from Alberti *et al.*, 2007). (a) – Some details of the mating sequence from above to below: *S. delamarei* male (black) has captured a female with its forelegs and deposits a rather large spermatophore (arrow). The male then turns round and impales the female onto the spermatophore. The spermatophore almost fills the "entire" female. The male has to separate the upper part of the spermatophore (which is more or less in the female) from the lower part which is attached to the ground. (b) – Longitudinal section of spermatophore of *S. delamarei*. Note the considerable amount of secretion forming the spermatophore and the rather small sperm chamber containing dense secretion and many synspermia. (c) – Spermatophore of *Rh. lootsi* drawn to same scale as that of *S. delamarei*. The head is (largely) represented by one synspermium. Stalk added schematically. Scale bar for b and c: 50 μm. (d) – Mating sequence seen in *Rh. lootsi*. Note that the spermatophore (arrow) produced by the male is considerably smaller bearing a very small head (i.e., mainly the synspermium). The male can use the same stalk several times depositing further synspermia on it. The male inserts its dorsoanteriad protruding processus into the female's genital opening prior to spermatophore deposition (not shown; see Coineau *et al.*, 2006 for more details).
Abbr.: SpC, sperm chamber.

regarded previously to be of the "primitive" droplet type, show a remarkable diversity and structural complexity, which exceeds at least in some species that of the studied Saxidromidae. Hence, it may be justified to classify even the spermatophore of *S. delamarei*, which is the most complex of the family, as rather simple.

In contrast, the sperm cells and the mating behaviour are certainly much derived and complex. In all three species, synspermia are produced. These peculiar sperm aggregates were until recently only known from few haplogyne spiders and comprise "fused" sperm enclosed in a common secretion sheath (Alberti and Weinmann, 1985; Alberti, 2000). In fact, it was shown for spiders, that the spermatids do not separate at the end of spermiogenesis (Michalik *et al.*, 2004) as is the case in Saxidromidae. In any case, Saxidromidae represent only the second taxon in the animal kingdom known to possess synspermia (Alberti *et al.*, 2007). Each synspermium contains a number of nuclei, acrosomal complexes and, in the case of spiders, axonemata in a common cytoplasm. Acari have aflagellate sperm cells and Actinotrichida have condensed nuclear material which has lost the nuclear envelope during spermatogenesis (hence called chromatin bodies) (Alberti 1980a, b, 1991, 2000, 2006; Alberti and Coons, 1999; Coons and Alberti, 1999). Accordingly, synspermia of Saxidromidae do not contain axonemata and possess chromatin bodies.

It may be mentioned again, that these clearly apomorphic peculiarities (testis histology, aflagellate sperm, chromatin bodies, chromatin body penetrated by the acrosomal filament) are shared only with Solifugae amongst other Arachnida (Alberti 1980a, b, c; Alberti 2000; Alberti 2006; Alberti and Peretti 2002; Klann *et al.*, 2005, 2009). Anactinotrichid mites (and other Arachnida) are distinctly different (Alberti 1980a, b, 1991, 2000, 2006). Unfortunately, these obvious results were recently incorrectly referred to by Shultz (2007). Interestingly, however, the idea of a sister group relationship of Actinotrichida and Solifugae has recently received a new molecular support (Dabert *et al.*, in press). It may be necessary in this context to state, that the synspermia of Saxidromidae and certain spiders without doubt evolved convergently.

The mating behaviour of these mites has been interpreted in terms of securing sperm priority and/or economics (Coineau *et al.*, 2006). The mites live in crevices of rocks exposed to the bright sunshine. They mate outside the crevices and thus the time for mating activities is limited to a short period during daytime. This favours competition for females, which in turn favours the evolution of the peculiar mating behaviour. Competition amongst males for females is evident in all three species (see also Coineau and Kovoor, 1982). Whereas in *S. delamarei* males produce a rather conventional spermatophore, both the other species have reduced the amount of secretion needed, which might save energy. Also, the number of sperm produced is much reduced.

The biological function (selective advantage) of the peculiar, certainly apomorphic, synspermia of these mites is still enigmatic (see below). With regard to the spiders, it was suggested that it may be more economic to produce synspermia than aggregates of isolated sperm surrounded by a common sheath (coenospermia) or single sperm each provided with a secretion layer (cleistospermia) and thus saving material used for the (many) secretion layers (Alberti 2000; Michalik *et al.*, 2004). But since *S. delamarei* produces a rather large spermatophore also containing synspermia, this interpretation is not really conclusive with respect to these mites.

Thus a scenario "explaining" the evolution of synspermia in Saxidromidae may be described in the following way:

1. Actinotrichida likely have indirect spermatophore transfer as the plesiotypic insemination type. This is one mode of sperm transfer securing internal fertilization which is necessary in terrestrial habitats. It may be modified within several taxa in various ways (with or without mating, semidirect sperm transfer with gonopods, direct sperm transfer with penis = perhaps modified spermatopositor) (e.g., Pauly, 1952; Taberly, 1957; Schuster, 1962; Schuster and Schuster, 1966, 1977; Alberti, 1974; Witte, 1975, 1991; Ehrnsberger, 1977; Schaller, 1979; Alberti *et al.*, 1991; Fernandez *et al.*, 1991; Norton *et al.*, 1993; Oldfield and Michalska, 1996; Alberti and

Coons, 1999; Walter and Proctor, 1999; Alberti and Michalik, 2004; Alberti and Schuster, 2005; Krantz and Walter, 2009).

2. Saxidromidae have kept the plesiotypic transfer mode, but have modified it considerably.

The male manipulates the female almost by brute force (securing sperm transfer) likely as an adaptation to the adverse habitat (heat, drought) and the resulting high competition for females (limited time due to increasing insolation). Coping with this, *S. delamarei* on the one hand and *B. roussouwi* and *R. lootsi* on the other, apparently evolved two different strategies:

a. *S. delamarei* during one mating event transfers relatively many synspermia, along with a long mating time and a large spermatophore (blocking almost the entire female genital tract) thus securing sperm priority by hindering further couplings and inseminations. However, in case of disturbances through other males, the mating male may be prevented from separating the spermatophore from the substrate which is necessary since the female is not able to do that and will die if the male fails to do this successfully (Coineau and Kovoor 1982). Hence, in this strategy the male may loose the entire invested material (many sperms, much spermatophore secretion) upon disturbance.

b. Both the other species transfer only one relatively small synspermium at a time. Thus at least some genetic material of the male will be transferred relatively fast. In case of disturbance, only a small amount of material is lost for the male (if it is not kept by the female) and the female can free itself from the tiny spermatophore stalk. If there is no disturbance, the male can deposit further synspermia on the same stalk thus transferring further synspermia. The sperm transfer thus is more secure, more effective and more economic. It may be speculated whether these mites are able to test the female's fertilization state or to remove sperm of previous males with their anterior protuberances thus making their activities even more effective. It seems conclusive to consider the thick secretion layers covering these synspermia (in contrast to those of *S. delamarei*) serve as a protective layer preventing damage caused through such actions by a subsequent male. (It may even be considered whether the thick layer found in *Rh. lootsi* is a protective correlate to the long unpaired processus found in this species).

The testes of all three species apparently contain rather few sperm (compared to other actinedid mites), with the South African species showing an extreme reduction. Evidently, the reduction in sperm and secretion invested by the males, and thus an increase in economy, is possibly due to the complex, effective and secure mating behaviour.

But why synspermia? Since we found synspermia in all three species, the suggested reduction of secretion material alone makes no sense, since the spermatophore of *S. delamarei* requires a lot of secretion as do the conspicuous secretion layers around the synspermia of the South African species. We suggest instead, that the "invention" of synspermia is a means of shortening spermiogenesis and thus is "cheaper" for the male. The latest steps of spermiogenesis are transferred into the female and hence occur only in the case of successful mating. Hence, the synspermia, which are so rarely found in the animal kingdom, may be a further step in making the sperm transfer procedure more economic for the male.

Further studies, in particular of fertilized females, are needed to better understand this peculiar sperm transfer. Such studies are currently being conducted.

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REFERENCES

Alberti G. 1974 — Fortpflanzungsverhalten und Fortpflanzungsorgane der Schnabelmilben (Acarina: Bdellidae, Trombidiformes) — Z. Morphol. Tiere, 78: 111-157.

Alberti G. 1979 — Fine structure and probable function of genital papillae and Claparède organs of

Actinotrichida. — In: Rodriguez J. (Ed). Recent advances in acarology, Vol. II. New York: Academic Press. p. 501 507.

Alberti G. 1980a — Zur Feinstruktur der Spermien und Spermiocytogenese der Milben (Acari): I. Anactinotrichida — Zool. Jahrb. Anat., 104: 77-138.

Alberti G. 1980b — Zur Feinstruktur der Spermien und Spermiocytogenese der Milben (Acari). II. Actinotrichida — Zool. Jahrb. Anat., 104: 144-203.

Alberti G. 1980c — Zur Feinstruktur des Hodenepithels und der Spermien von *Eusimonia mirabilis* Roewer, 1934 (Solifugae, Arachnida) — Zool. Anz., 204: 345-352.

Alberti G. 1991 — Spermatology in the Acari: Systematic and functional implications. — In: Schuster R., Murphy, P. W. (Eds). The Acari: reproduction, development and life history strategies. London: Chapman & Hall. p. 77-105.

Alberti G. 2000 — Chelicerata. — In: Jamieson B. G. M. (Ed). Progress in male gamete ultrastructure and phylogeny. In: Adiyodi K. G., Adiyodi R. G. (Eds). Reproductive biology of the invertebrates. Vol. 9, p.B. New Delhi: Oxford & IBH Publishing/Wiley: NewYork. p. 311-388.

Alberti G. 2006 — On some fundamental characteristics in acarine morphology — Atti Accad. Naz. Ital. Entomol. R. A. LIII - 2005: 315-360.

Alberti G., Coons L. B. 1999 — Acari - Mites. — In: Harrison F. W. (Ed). Microscopic anatomy of invertebrates. Vol. 8c. New York: Wiley-Liss. p. 515-1265.

Alberti G., Weinmann C. 1985 — Fine structure of spermatozoa of some labidognath spiders (Filistatidae, Segestriidae, Dysderidae, Oonopidae, Scytodidae, Pholcidae; Araneae; Arachnida) with remarks on spermiogenesis — J. Morphol., 185: 1-35.

Alberti G., Fernandez N. A., Kümmel G. 1991 — Spermatophores and spermatozoa of oribatid mites (Acari: Oribatida). Part II: Functional and systematical considerations — Acarologia, 32: 435-449.

Alberti G., Fernandez N. A., Coineau Y. 2007 — Fine structure of spermiogenesis, spermatozoa and spermatophore of *Saxidromus delamarei* Coineau, 1974 (Saxidromidae, Actinotrichida, Acari) — Arthropod Struct. & Dev., 36: 221-231.

Alberti G., Nuzzaci G. 1996 — SEM and TEM Techniques. — In: Lindquist E. E., Bruin J., Sabelis M. W. (Eds). Eriophyoid mites. Their biology, natural enemies and control. World crop pests. Vol. 6. Amsterdam: Elsevier Sci. Publ. p. 399-410.

Alberti G., Peretti A. 2002 — Fine structure of male genital system and sperm in Solifugae does not support a sister-group relationship with Pseudoscorpiones (Arachnida) — J. Arachnol., 30: 268-274.

Alberti G., Schuster R. 2005 — Behavioural and ultrastructural peculiarities of reproduction in *Collohmannia gigantea* (Oribatida: Mixonomata). — In: Weigmann G., Alberti G., Wohltmann A., Ragusa S. (Eds). Acarine biodiversity in the natural and human sphere. Phytophaga (Palermo), 14 (2004): 129-140.

Coineau Y. 1976 — Les parades sexuelles des Saxidrominae Coineau 1974 (Acariens Prostigmates, Adamystidae) — Acarologia, 18: 234-240.

Coineau Y. 1979 — Les Adamystidae, une étonnante famille d'Acariens prostigmates primitifs. — In: Piffl E. (Ed). Proceedings of the 4th International Congress of Acarology, 1974. Budapest: Akadémiai Kiadó. p. 431-435.

Coineau Y., Kovoor J. 1982 — *Saxidromus delamarei* (Acarines prostigmate primitif) - aspects biologiques évolutifs. Réalisateur A. R. Devec — Service du Film de Recherche Scientifique. 96 Bd. Raspail, 75272 Paris Cedex 06 France; 21 min., French and English versions, 16mm and VHS Pal.

Coineau Y., Théron P. D., Fernandez N. 2006 — Parades et dimorphismes sexuels comparés chez deux nouveaux genres de Saxidromidae (Acari, Alycina) d'Afrique du Sud — Acarologia, 46: 65-87.

Coons L. B., Alberti G. 1999 — Acari - Ticks. — In: Harrison, F. W. (Ed.) Microscopic Anatomy of Invertebrates. Vol. 8b. New York: Wiley -Liss. p. 267-514.

Dabert M., Witalinski W., Kazmierski A., Olszanowski Z., Dabert J. 2010 in press — Molecular phylogeny of acariform mites (Acari, Arachnida): strong conflict between phylogenetic signal and long-branch attraction artifacts — Mol. Phylogenetic Evol., doi: 10.1016/j.ymper.2009.12.020.

Ehrnsberger R. 1977 — Fortpflanzungsverhalten der Rhagidiidae (Acarina, Trombidiformes) — Acarologia, 19: 67-73.

Ehrnsberger R. 1988 — Mating behaviour of *Linopodes* sp. (Acariformes: Eupodoidea). — In: Channabasavanna G. P., Viraktamath, C. A. (Eds). Progress in Acarology. Vol. 1. New Delhi: Oxford and IBH Publ. p. 211-218.

Evans G. O. 1992 — Principles of acarology — Wallingford: C.A.B International. pp. 563.

Fernandez N. A., Alberti G., Kümmel G. 1991 — Spermatophores and spermatozoa of oribatid mites (Acari: Oribatida). Part I: Fine structure and histochemistry — Acarologia, 32: 261-286.

Klann A. E., Peretti A. V., Alberti G. 2005 — Ultrastructure of male genital system and spermatozoa of a Mexican camel-spider of the *Eremobates pallipes* speciesgroup (Arachnida, Solifugae) — J. Arachnol., 33: 613-622.

Klann A. E., Bird T., Peretti A. V., Gromov A. V., Alberti G. 2009 — Ultrastructure of spermatozoa of solifuges

(Arachnida, Solifugae): Possible characters for their phylogeny? — Tissue & Cell, 41: 91-103.

Krantz G. W., Walter D. E. (Eds). 2009 — A manual of acarology. 3rd ed. — Texas Tech University Press: Lubbock. pp. 807.

Michael A. D. 1896 — The internal anatomy of *Bdella* — Trans. Linn. Soc. Lond. (ser. 2), 6: 477-528.

Michalik P., Dallai R., Giusti F., Alberti G. 2004 — The ultrastructure of the peculiar synspermia of some Dysderidae (Araneae, Arachnida) — Tissue & Cell, 36: 447-460.

Norton R. A., Kethley J. B., Johnston D. E., OConnor B. M. 1993 — Phylogenetic perspectives on genetic systems and reproductive modes of mites. — In: Wrensch D. L., Ebbert M. A. (Eds.). Evolution and diversity of sex ratio in insects and mites. New York: Chapman and Hall. p. 8-99.

Oldfield G. N., Michalska K. 1996 — Spermatophore deposition, mating behavior and population mating structure. — In: Lindquist E. E., Sabelis M. W., Bruin J. (Eds). Eriophyoid Mites - Their biology, natural enemies and control. Amsterdam: Elsevier. p. 185-198.

Pauly F. 1952 — Die "Copula" der Oribatiden (Moosmilben) — Naturwiss., 39: 572-573.

Reynolds E. S. 1963 — The use of lead citrate at high pH as electron opaque stain in electron microscopy — J. Cell Biol., 17: 208-212.

Richardson K.C., Jarrett L. J., Finke E. H. 1960 — Embedding in epoxy resins for ultrathin sectioning in electron microscopy — Stain Technol., 35: 313-323.

Schaller F. 1979 — Significance of sperm transfer and formation of spermatophores in arthropod phylogeny. — In: Gupta A. P. (Ed). Arthropod phylogeny. New York: Van Nostrand Reinhold Comp., p. 587-608.

Schuster R. 1962 — Nachweis eines Paarungszeremoniells bei den Hornmilben (Oribatei, Acari) — Naturwiss., 49: 502-503.

Schuster R., Schuster I. J. 1966 — Über das Fortpflanzungsverhalten von Anystiden Männchen (Acari, Trombidiformes) — Naturwiss., 53: 162.

Schuster R., Schuster I. J. 1977 — Ernährungs und fortpflanzungsbiologische Studien an der Milbenfamilie Nanorchestidae (Acari, Trombidiformes) — Zool. Anz., 199: 89-94.

Shultz J. 2007 — A phylogenetic analysis of the arachnid orders based on morphological characters — Zool. J. Linn. Soc., 150: 221-265.

Spurr A. R. 1969 — A low-viscosity epoxy resin embedding medium for electron microscopy — J. Ultrastruct. Res., 26: 31-43.

Taberly, G. 1957 — Observations sur les spermatophores et leur transfert chez les Oribates (Acariens) — Bull. Soc. ent. France, 82: 139-145.

Van der Hammen L. 1980 — Glossary of Acarological Terminology. Vol. I. General Terminology — The Hague: Dr. W. Junk Publ. pp. 244.

Walter D., Proctor H. 1999 — Mites. Ecology, Evolution and Behaviour — Wallingford: CAB International. pp. 322.

Witte H. 1975 — Funktionsanatomie der Genitalorgane und Fortpflanzungsverhalten bei den Männchen der Erythraeidae (Acari, Trombidiformes) — Z. Morphol. Tiere, 80: 137-180.

Witte H. 1991 — Indirect sperm transfer in prostigmatic mites from a phylogenetic viewpoint. — In: Schuster R., Murphy P. W. (Eds.). The Acari: Reproduction, Development and Life history Strategies. London: Chapman & Hall. p. 137-176.

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