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# Spermiogenesis in *Psilochorus simoni* (Berland, 1911) (Pholcidae, Araneae): Evidence for considerable within-family variation in sperm structure and development

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## Abstract

A large number of characters and considerable variation among taxa make animal sperm cells promising objects for phylogenetic studies. However, our knowledge about sperm structure and development in spiders is still rudimentary. In pholcids, previous studies of two species representing different subfamily level taxa have revealed conspicuous differences. Here, we report on a representative of a third subfamily level taxon, confirming substantial variation in sperm structure and development within the family. The male genital system in *Psilochorus simoni* (Berland, 1911) consists of paired testes and deferent ducts which lead into a common ejaculatory duct. The somatic cells of the testes show a high secretory activity, and produce at least two different kinds of secretion. The spermatozoa show features already known from other Pholcidae as well as unique characters. The acrosomal vacuole is tube-like with a narrow subacrosomal space. The axoneme migrates deep into the nucleus and is finally located near the acrosomal vacuole. Thus, the postcentriolar elongation of the nucleus is very long. A centriolar adjunct is not present and after the coiling process the implantation fossa is completely filled with glycogen which is also found in larger amounts within the cytoplasm of the sperm cell. After the coiling process, a vesicular area is present that becomes most prominent in the periphery of the sperm cell and surrounds the axoneme and parts of the nucleus. The secretion sheath surrounding the mature spermatozoon is already formed in the lumen of the testis, possibly by a secretion present in the testis but absent in the deferent duct. Sperm are transferred as cleistospermia. Results are compared with previous studies on pholcid spermiogenesis and sperm structure.


***Keywords:*** Sperm; Phylogeny; Secretion; Vesicular area; Cleistospermia

## Introduction

The study of sperm structure and development is providing a wealth of new data relevant for phylogenetic inference (e.g., Dallai et al., 2003a, b; Scheltinga et al., 2003; Marotta and Ruhberg, 2004; Meisner et al., 2005). Since the first ultrastructural study on spider spermatozoa by Ōsaki (1969), 44 species of 17 families have been observed more or less in detail (summary in Alberti, 2000; Michalik et al., 2003, 2004a, b, 2005a, b, in press). Spider spermatozoa are always coiled-flagellate cells that are encapsulated in a secretion sheath. Details of this encapsulation mirror high level phylogenetic

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patterns: early derivative spider groups like Mesothelae and Mygalomorphae have coenospermia (several single sperm cells surrounded by a common secretion sheath). In contrast, Entelegynae have cleistospermia (each single sperm cell surrounded by a secretion sheath) (summary in Michalik et al., 2004a). Likewise, spermiogenesis and sperm structure promise to provide useful characters at low taxonomic levels. For example, a comparative study of several tetragnathid spiders (Michalik et al., in press) has shown considerable differences in the organization of spermatozoa at genus-level within a family. However, data on spider sperm ultrastructure and development continue to be fragmentary in most groups.

In pholcids, only two species have been studied in detail: *Pholcus phalangioides* Fuesslin, 1775 (Rosati et al., 1970; Alberti and Weinmann, 1985; Michalik and Uhl, 2005) and *Holocnemus pluchei* Scopoli, 1763 (Lopez and Boissin, 1976; Michalik et al., 2005a). These studies revealed considerable variation within the family, regarding for example the acrosomal vacuole, conformation of nuclear material and shape of the proximal centriole. Interestingly, these two species have been assigned to different subfamily level taxa: ''pholcines'' and ''holocnemines'' (Huber, 2000; Bruvo-Madarić, 2005). To evaluate the potential of sperm characters for phylogenetic inference, investigations on further Pholcidae are needed.

In the present study, we describe the male genital system and spermatozoa of the small pholcid spider *Psilochorus simoni,* a synanthropic species introduced from the New World and dispersed over most parts of Europe (Fürst and Blandenier, 1993; Huber, 1994). This species has been assigned to a different subfamily level taxon, the ''New World clade'' (Huber, 2000). We give a detailed account of spermiogenesis and compare our results with data on *P. phalangioides* and *H. pluchei*.

## Material and methods

Adult males of *P. simoni* Berland, 1911 were collected in houses in Bonn (Germany).

### Transmission electron microscopy (TEM)

Three male specimens were dissected in 0.1 M phosphate buffer to which 1.8% sucrose was added. The isolated genital systems were fixed in 2.5% glutaraldehyde in the same buffer followed by postfixation in buffered 2% osmium tetroxide. After rinsing, the tissue pieces were dehydrated in graded ethanols and embedded in Spurr's resin (Spurr, 1969). Ultrathin sections were made with a Leica ultramicrotome and stained with uranyl acetate and lead citrate (Reynolds, 1963). Examination was performed with a Zeiss EM 10A electron microscope.

### Scanning electron microscopy (SEM)

The isolated male genital systems of three males were split open in a droplet of phosphate buffer (see above) using thin needles onto glass coverslips covered with 1% poly L-lysin. After 10 min sedimentation the adhering material was fixed with 2.5% glutaraldehyde in buffer for 1 h at 4 °C. Samples were then rinsed in buffer and postfixed in buffered 1% osmium tetroxide, dehydrated in graded ethanols, dried in a BAL-TEC CPD 030 critical point dryer using amylacetate as intermedium, coated with gold-palladium in a Quorum Technologies SC7620 sputtering device and examined in a Leo DSM 940A scanning electron microscope.

## Results

### Spermiogenesis

After the meiotic divisions the spermatids start to differentiate. They are connected via extensive cell bridges and their cytoplasm becomes more electron-lucent. In the following the main developmental stages of spermiogenesis are described.

#### Early spermatids

At the anterior pole of the early spermatid, an acrosomal vacuole is formed (Figs. 1a, b, d). It is produced by fusion of vesicles of the Golgi apparatus located beside the anterior pole of the nucleus (Fig. 1b). The acrosomal vacuole is anteriorly in contact with the cell membrane and posteriorly delimited against the nucleus by a layer of electron-dense material (Fig. 1b). The vacuole is indented posteriorly forming a subacrosomal space which contains the acrosomal filament (Figs. 1a, b). This filament leads into the nuclear canal which runs through the nucleus to its posterior end (Figs. 1a–d, see also below).

At the posterior pole of the spermatid, the two centrioles of the axoneme are arranged in a tandem position and migrate towards the nucleus (Fig. 1a). Before this migration takes place, they are orientated perpendicular to each other. The axoneme originates from the distal centriole and becomes very long even in these early spermiogenic stages (~45 μm; Fig. 1e). As a result of the migration of the axoneme, the plasmalemma is invaginated to form a flagellar tunnel (Figs. 1a, c, d). The axoneme has a $9 \times 2 + 3$ microtubular pattern (see Fig. 6d).

Simultaneously to the migration of the axoneme, the nucleus is indented in front of the axonemal basis

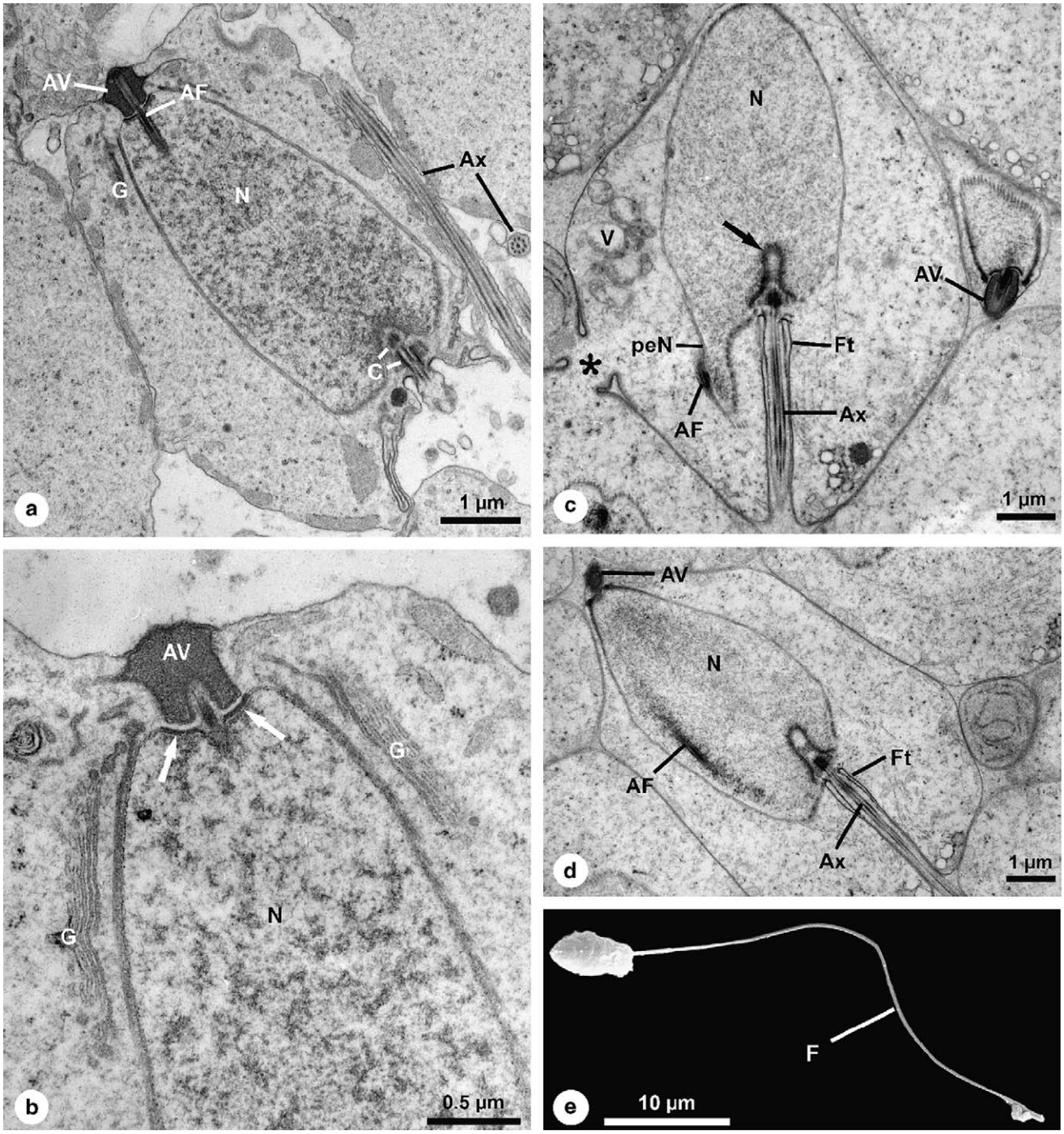



**Fig. 1.** Early spermatids of *P. simoni*. (a) Longitudinal section of a spermatid with the acrosomal vacuole and filament at the anterior pole of the nucleus and the axonemal basis located posteriorly. (b) The acrosomal vacuole is formed by fusion of Golgi vesicles originating from the Golgi apparatus close to the nucleus. Note the plate of dense material which borders the vacuole (arrows). (c) The implantation fossa is bordered by dense material (arrow) and the nucleus starts to elongate on one side behind the axonemal basis. As a result of the migration of the axoneme towards the nucleus a flagellar tunnel is formed. Note the cell bridge connecting the spermatids (asterisk); the acrosomal vacuole at the right belongs to a different spermatid. (d) The acrosomal filament extends into the periphery of the nucleus (cf. Fig. 2a). (e) Note the long flagellum in contrast to the cell body. *Abbreviations*: AF, acrosomal filament; AV, acrosomal vacuole; Ax, axoneme; C, centriole; F, flagellum; Ft, flagellar tunnel; G, Golgi apparatus; N, nucleus; peN, postcentriolar elongation of the nucleus; V, vesicle.

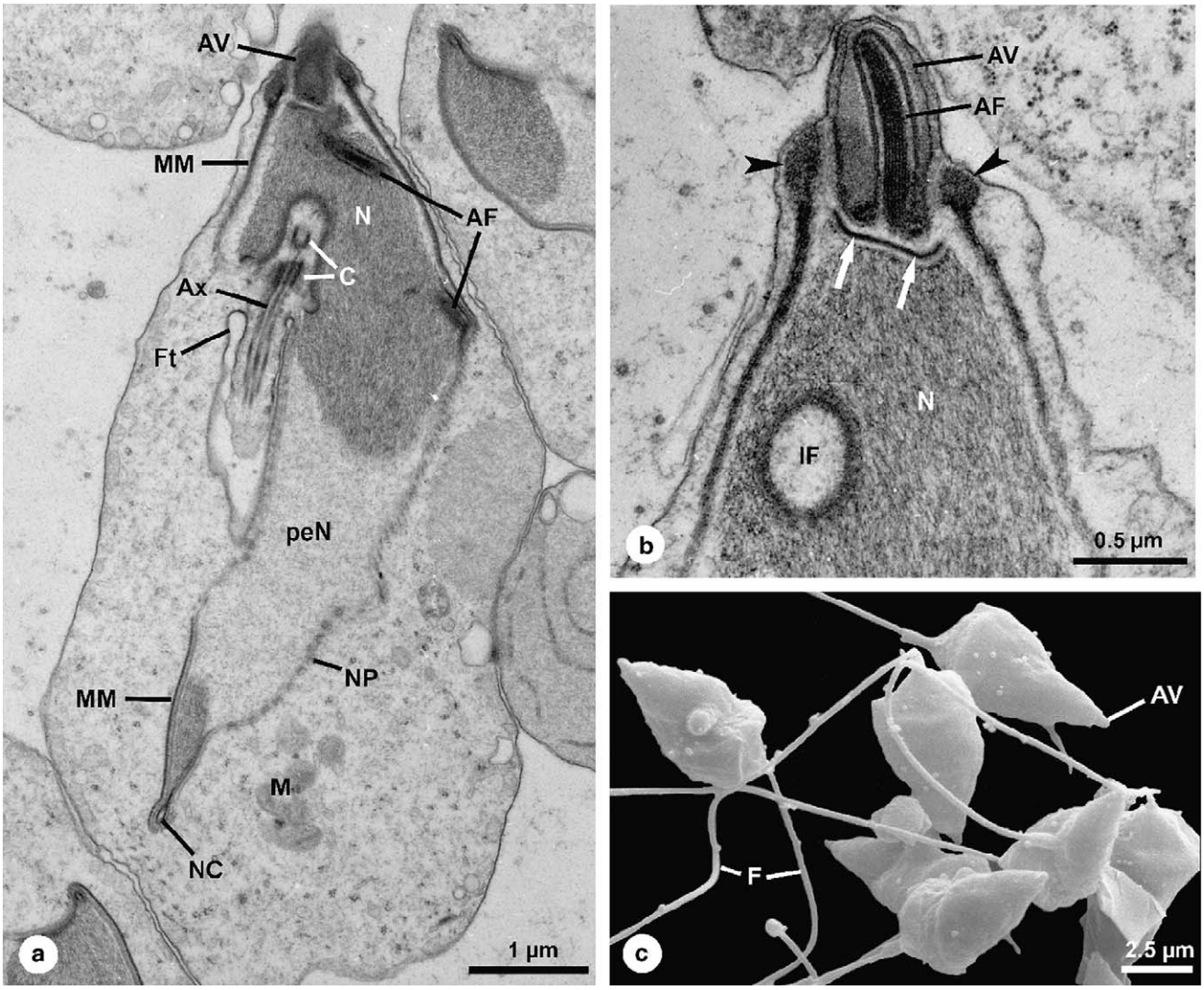


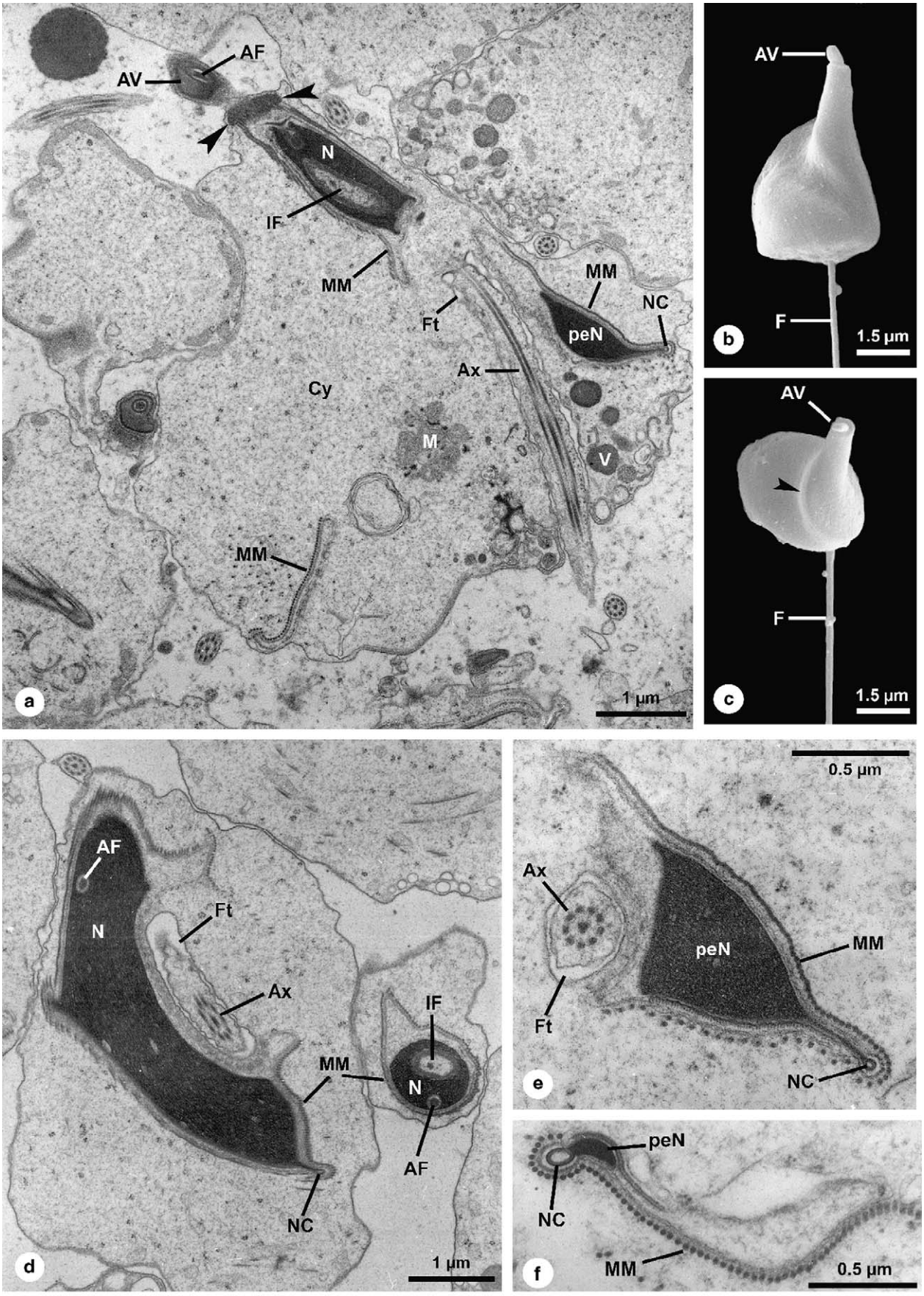

**Fig. 2.** Mid-spermatids of *P. simoni*. (a) Longitudinal section of a spermatid. The axoneme migrates deeply into the nucleus and is already close to the acrosomal vacuole. The nucleus strongly elongates in an asymmetrical way. Note the electron-lucent parts of the nucleus and the acrosomal filament which does not extend to the posterior end of the nucleus. (b) The acrosomal vacuole starts to elongate and is posteriorly delimited by a dense plate (arrows). Note the dense ring close to the acrosomal vacuole (arrowheads, see also Fig. 3a). The implantation fossa does not contain a centriolar adjunct or other material. (c) The mid-spermatids have a cone-shape appearance resulting from the elongation and transformation of the main cell components. *Abbreviations*: AF, acrosomal filament; AV, acrosomal vacuole; Ax, axoneme; C, centriole; F, flagellum; Ft, flagellar tunnel; IF, implantation fossa; M, mitochondria; MM, manchette of microtubules; N, nucleus; NC, nuclear canal; NP, nuclear pores; peN, postcentriolar elongation of the nucleus.

forming the so-called implantation fossa which is bordered by dense material (Figs. 1c, d). Furthermore, the nucleus starts to elongate and a postcentriolar elongation is formed on one side. Thus, the nucleus assumes an asymmetrical shape (Fig. 1c).

### Mid spermatids

In mid-stages of spermiogenesis, the acrosomal vacuole starts to elongate and is posteriorly surrounded by a ring of dense material (Figs. 2a, b, 3a, 4a, arrowheads). The vacuole is slightly sunken into the nucleus and delimited by a band of dense material (Fig. 2b, arrows). The subacrosomal space deeply indents the whole vacuole and the acrosomal filament leads into the peripheral nuclear canal. However, the filament does not reach the end of the nuclear canal (Fig. 2a).

The chromatin of the nucleus starts to condensate and assumes a fibrillar appearance (Figs. 2a, b). Within the elongated nucleus, electron-lucent and electron-dense areas are present (Fig. 2a). Those parts of the nucleus which contain condensed chromatin are surrounded by a dense manchette of microtubules; in these areas, no


AF
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nuclear pores appear in the nuclear envelope (Fig. 2a). In contrast, those areas of the nuclear envelope not covered by the manchette of microtubules show numerous pores (Figs. 2a, 3d).

During spermiogenesis, the centrioles migrate deeply into the implantation fossa and are finally located close to the acrosomal vacuole (Figs. 2a, b). As a result of this, the postcentriolar elongation is the largest part of the nucleus (Fig. 2a). No material was observed within the implantation fossa (Fig. 2b).

The slightly elongated appearance of the spermatids is seen in Fig. 2c. Due to the elongation of nucleus and acrosomal vacuole the spermatids become cone-shaped with larger masses of cytoplasm at their posterior ends (cf. Fig. 2a).

### Late spermatids

The late stages of spermiogenesis are mainly characterized by the condensed chromatin of the nucleus which now has an electron-dense appearance (Figs. 3a, d–f, 4a–c). The elongated acrosomal vacuole protrudes from the main cell body (Figs. 3b, 4d, e). The acrosomal filament is very short and ends behind the axonemal basis leaving the nuclear canal empty (Figs. 3a, d, 4a). Finally, the acrosomal vacuole has a long tube-like shape and is posteriorly surrounded by a ring of dense material (Fig. 4a, cf. Fig. 3a). The manchette of microtubules is still present (Figs. 4a–c).

The electron-lucent areas within the nucleus are very small and are only present close to the axoneme (Figs. 3a, e, 4c). The long postcentriolar elongation of the nucleus coils in a wide loop around the axoneme and becomes narrow posteriorly (Figs. 3a, c, 4c–e). Anteriorly it is triangular in cross-section. The microtubules surrounding the nucleus are more densely arranged on the outer (convex) periphery of it (Figs. 3a, e, f). The implantation fossa is delimited by dense material and contains few distinct patches (Figs. 3a, d, 4b). The length of the axoneme at the end of spermiogenesis is ~50 μm (Fig. 4d).

Prior to coiling, the spermatids are still connected by small cell bridges. Within the cytoplasm, additional cell components like vesicles and mitochondria are present (Figs. 3a, 4c).

After the coiling process, a vesicular area within the spermatids is evident (Fig. 5a). The nucleus coils twice and the axoneme turns in the periphery of the cell (Fig. 5a). The manchette of microtubules is reduced during the coiling process and is then no longer present (Fig. 5a). The implantation fossa is filled with glycogen which is also present in the cytoplasm and partly accompanies the postcentriolar elongation of the nucleus. Mitochondria and membranes are present (Fig. 5a). The spermatids become compact after coiling and are transferred into the lumen of the testis (Fig. 5b, c). Within the testis they are embedded in a homogenous secretion likely produced by the somatic cells. Furthermore, two kinds of secretion droplets can be distinguished (Fig. 5c, see above). The spermatids are surrounded by a secretion sheath while still within the lumen of the testis (Fig. 5d).

### Mature spermatozoa

During the very late stages of spermiogenesis the vesicular area becomes more extensive and finally surrounds the axoneme and parts of the nucleus in the periphery of the cell (Figs. 5d, 6c, d). Large amounts of glycogen are present in the cell and within the implantation fossa (Fig. 6c). Furthermore, membranous areas are present within the spermatozoa (Fig. 6d). The mature spermatozoa in the deferent duct and ejaculatory duct are embedded in a homogenous matrix and surrounded by only one kind of secretion droplet (Figs. 6a, b, see below).

## Male genital system

The male genital system of *P. simoni* consists of paired testes and deferent ducts which lead into the unpaired ejaculatory duct. The testes are squat and bordered by the silk glands and the midgut gland. Within each testis different stages of spermatogenesis are present, with spermatids arranged in cysts which are surrounded by extensions of the somatic cells. The lumen of the testis is branched and sometimes bounded only by very thin extensions of somatic cells (Fig. 5b). Within the lumen, two kinds of secretion droplets are embedded in a homogenous matrix, together with the sperm cells: large amounts of small very electron-dense droplets and a few large droplets which are less electron-dense (Fig. 5c).

The deferent ducts are convoluted and filled with seminal fluid consisting of secretion and mature spermatozoa (Figs. 6a, b; see above). Their flat

**Fig. 3.** Late spermatids of *P. simoni*. (a) Longitudinal section of a spermatid. The acrosomal vacuole is posteriorly surrounded by a ring of dense material (arrowheads, cf. Figs. 2a, 4a). The nucleus coils around the axoneme and becomes very thin posteriorly (cf. Fig. 3f). (b–c) The spermatids are asymmetrical as a result of the peculiar elongation of the nucleus. The nuclear canal turns at the periphery of the nucleus (arrowhead). (d–f) Sections of the nucleus in different parts. The manchette of microtubules does not cover the nucleus in the region of the flagellar tunnel. The microtubules are denser at the outer side of the nucleus. *Abbreviations*: AF, acrosomal filament; AV, acrosomal vacuole; Ax, axoneme; Cy, cytoplasm; F, flagellum; Ft, flagellar tunnel; IF, implantation fossa; M, mitochondria; MM, manchette of microtubules; N, nucleus; NC, nuclear canal; peN, postcentriolar elongation of the nucleus; V, vesicle.

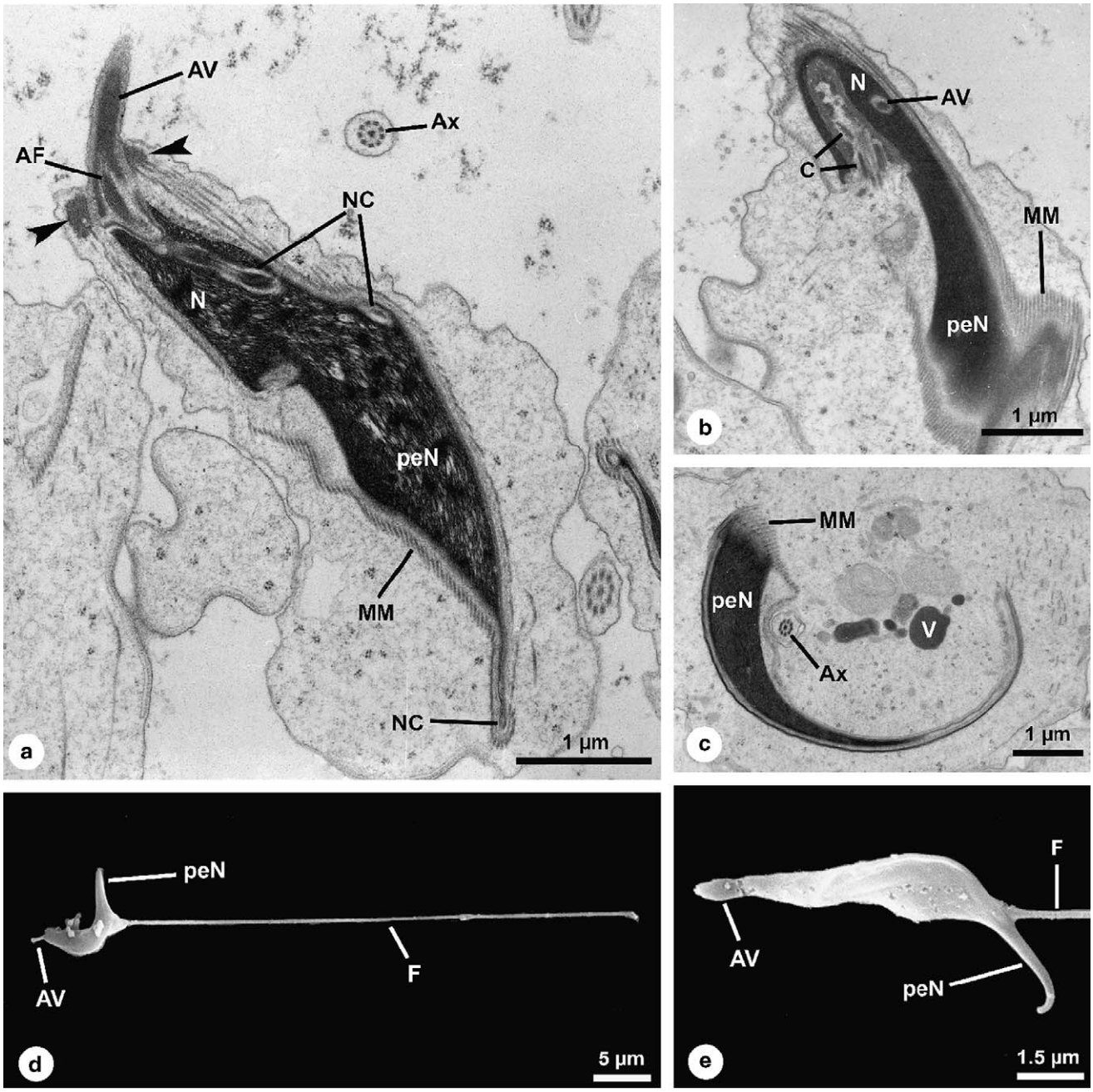


**Fig. 4.** Late spermatids of *P. simoni*. At the end of spermiogenesis, the acrosomal vacuole is elongated and tube-like shaped (a, d–e). The long postcentriolar elongation of the nucleus turns once around the axoneme (c, e) and the nuclear canal is located in the periphery of it (a). The acrosomal filament extends only into the anterior part of the nuclear canal (a). The implantation fossa is bordered by dense material and contains few patches of dense material (b). Note the proportion of flagellum and cell body (d). *Abbreviations*: AF, acrosomal filament; AV, acrosomal vacuole; Ax, axoneme; C, centriole; F, flagellum; IF, implantation fossa; MM, manchette of microtubules; N, nucleus; NC, nuclear canal; peN, postcentriolar elongation of the nucleus; V, vesicle.

epithelium is surrounded by a muscle layer (Fig. 6a). Apically, the epithelium bears microvilli and contains vesicles indicating secretory activity (Fig. 6a). The ejaculatory duct is located close to the genital opening and characterized by a wide lumen that is filled with seminal fluid composed of mature spermatozoa and large amounts of secretion (Fig. 6b).

## Discussion

### Male genital system

The male genital system of *P. simoni* in principle corresponds with the usual condition in spiders: paired testes lead into thin, convoluted deferent ducts that combine

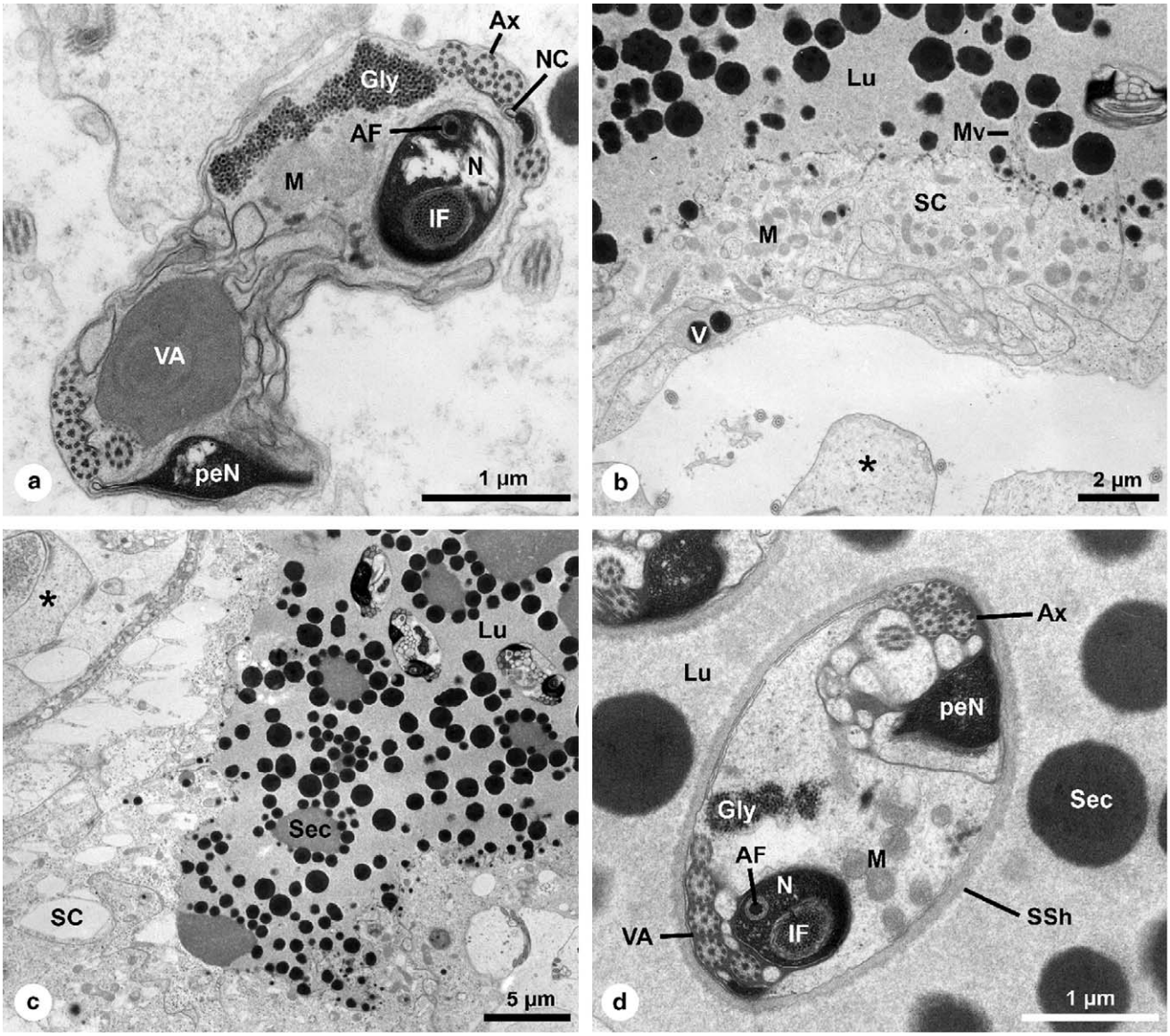


**Fig. 5.** Coiled spermatids and mature spermatozoa in the testis of *P. simoni*. (a) Coiled spermatid. The implantation fossa is now filled with glycogen which is also present in the cytoplasm. The axoneme turns four times in the periphery of the cell. Note the vesicular area. (b) The somatic cells bordering the lumen of the testis bear microvilli. Note the vesicles and mitochondria. Asterisk indicates a spermatid. (c) Within the lumen of the testis two different kinds of secretory droplets can be distinguished. (d) Mature spermatozoon in the lumen of the testis. Note the secretion sheath surrounding it. *Abbreviations*: AF, acrosomal filament; Ax, axoneme; Gly, glycogen; IF, implantation fossa; Lu, lumen; M, mitochondria; Mv, microvilli; N, nucleus; NC, nuclear canal; peN, postcentriolar elongation of the nucleus; SC, somatic cells; Sec, secretion; SSh, secretion sheath; V, vesicle; VA, vesicular area.

to form a wide ejaculatory duct (cf. Bertkau, 1875; Gerhardt and Kaestner, 1937/38; Crome, 1951; Kim et al., 1993; Knoflach, 1998; Michalik and Uhl, 2005). However, the genital system of *P. simoni* differs in shape compared to that of other pholcid spiders studied. The testes are small and compact, in contrast to the long tube-like testes known from *P. phalangioides* and *H. pluchei* (Michalik et al., 2005a; Michalik and Uhl, 2005). Crome (1951) suggested that the shape of the male genital system correlates with the general organization of the opisthosoma. This is a plausible explanation in pholcids since the roundish opisthosoma of *P. simoni* is very different from the elongated opisthosomata of *P. phalangioides* and *H. pluchei*.

## Spermiogenesis and spermatozoa

The spermatozoa of *P. simoni* show several conspicuous characters which have not yet been reported from other pholcids or other Haplogynae (sensu Coddington and Levi, 1991). At the same time, they show characters similar to those of other pholcid spiders (see Table 1).

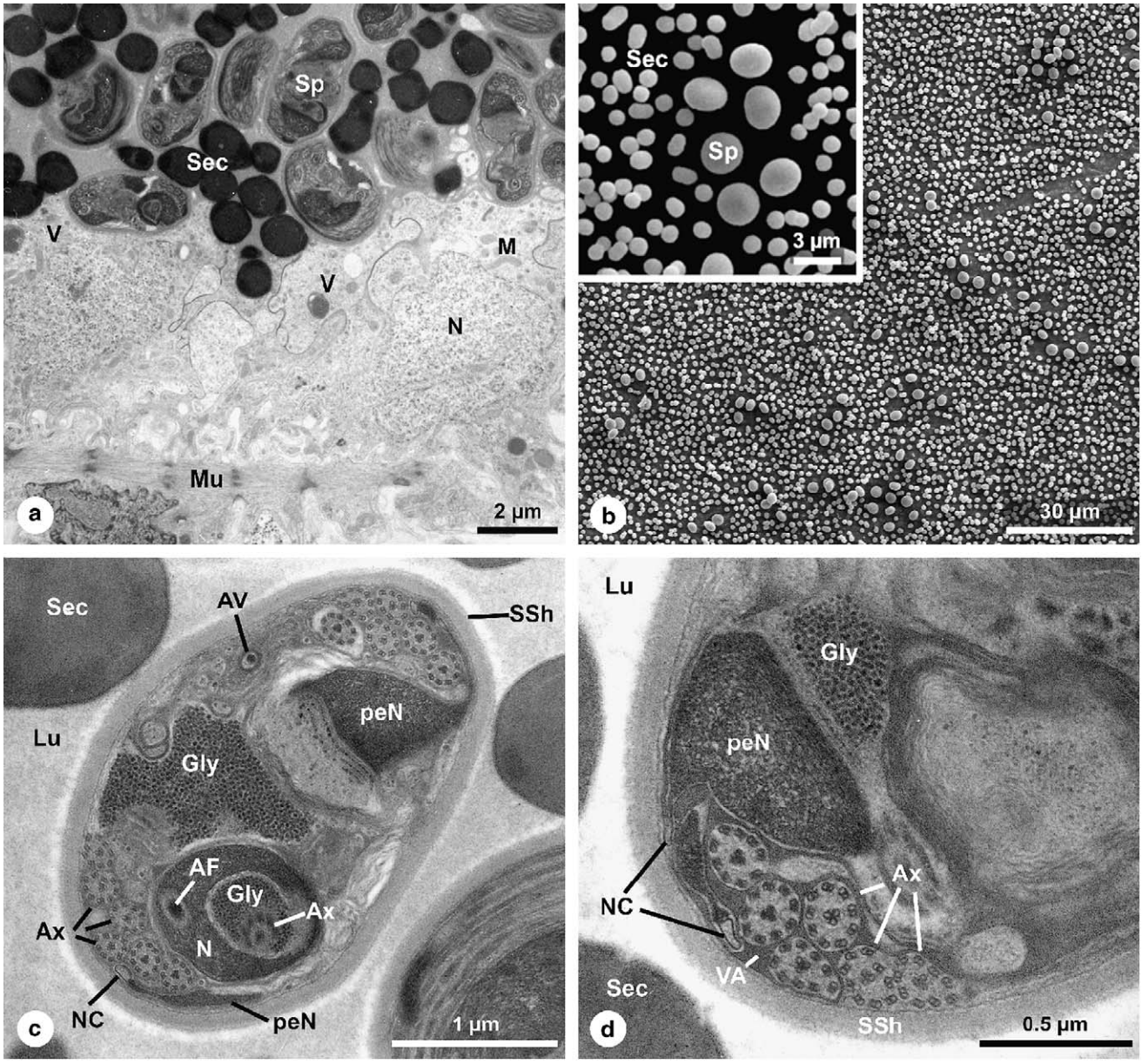


**Fig. 6.** Deferent duct, spermatozoa and seminal fluid of *P. simoni*. (a) The flat epithelium of the deferent duct is bordered by a muscle layer. In the lumen only one kind of secretory droplet is present. (b) The seminal fluid contains the spermatozoa and one kind of secretory droplets. Note the relation in the amount of sperm cells and secretion. (c) The mature spermatozoon contains large amounts of glycogen. It is surrounded by a thick secretion sheath. (d) Detail of the periphery of a spermatozoon. The vesicular area surrounds the axoneme and parts of the postcentriolar elongation of the nucleus. Note the $9 \times 2 + 3$ axonemal pattern. *Abbreviations*: AF, acrosomal filament; AV, acrosomal vacuole; Ax, axoneme; Gly, glycogen; Lu, lumen; M, mitochondria; Mu, muscle; N, nucleus; NC, nuclear canal; peN, postcentriolar elongation of the nucleus; Sec, secretion; Sp, spermatozoon; SSh, secretion sheath; V, vesicle; VA, vesicular area.

The following are characters found in all three pholcid species studied: (1) an elongated tube-like acrosomal vacuole; (2) the absence of a centriolar adjunct; (3) an implantation fossa that is filled with glycogen at the end of spermiogenesis; (4) cleistospermia as transfer units (Alberti and Weinmann, 1985; Lopez and Boissin, 1976; Michalik et al., 2005a; PM, personal observation).

Differences to *H. pluchei* were found with respect to (1) the acrosomal vacuole (*H. pluchei* shows parts with different electron density during spermiogenesis: Lopez and Boissin, 1976); (2) the absence of "inner microtubules" in the implantation fossa in early and mid-spermatids (found also in *Smeringopus* sp., PM, personal observation); (3) the types of secretory droplets in the seminal fluid (see below).

**Table 1.** Comparison of relevant sperm characters in Pholcidae

| | ***Psilochorus simoni*** (Berland, 1911) (present study) | ***Holocnemus pluchei*** (Scopoli, 1763) (Lopez and Boissin, 1976; Michalik et al., 2005a) | ***Pholcus phalangioides*** (Fuesslin, 1775) (Alberti and Weinmann, 1985; Michalik and Uhl, 2005) |
|---|---|---|---|
| ***Acrosomal complex*** | | | |
| Acrosomal vacuole (AV) | Elongated, tube-like shape | Elongated, tube-like shape | Elongated, tube-like shape with specializations |
| ***Nucleus*** | | | |
| Nuclear canal (NC) | Peripheral | Peripheral | Central, posteriorly displaced by IF |
| ***Postcentriolar elongation (peN)*** | Long | Normal | Short |
| Implantation fossa (IF) | Small | Large | Large |
| Implantation fossa filled with | Glycogen | Glycogen, ''inner microtubules'' during spermiogenesis | Glycogen |
| Helical band of nuclear material | Absent | Absent | Present |
| ***Axoneme*** | | | |
| Centriolar adjunct (ca) | Absent | Absent | Absent |
| Proximal centriole | Normal | Normal | Prolonged |
| Axonemal basis located | Near acrosomal vacuole | Posterior part of the nucleus | Posterior part of the nucleus |
| ***Transfer form*** (***cleistospermia***) | | | |
| Formation of the secretion sheath | Testis | Deferent duct | Deferent duct |
| Vesicular area (VA) | Present | Absent | Absent |
| ***Secretions*** | | | |
| Secretory droplets in seminal fluid | 1 type; 2 types in testes | 2 types | 3 types |

Differences to *P. phalangioides* concern (1) the acrosomal vacuole (*P. phalangioides* shows specializations: Alberti and Weinmann, 1985); (2) the absence of a helical band of nuclear material found in *P. phalangioides* (Alberti and Weinmann, 1985; present also in *Spermophora senoculata*, a species belonging to the same subfamily level taxon as *P. phalangioides*; PM, personal observations); (3) the nuclear canal (in *P. phalangioides* central and posteriorly displaced by the implantation fossa: Alberti and Weinmann, 1985); (4) the proximal centriole (prolonged in *P. phalangioides*: Alberti and Weinmann, 1985); (5) the types of secretory droplets in the seminal fluid (see below).

A unique character of the sperm cells of *P. simoni* is the deep nuclear indentation. The axonemal basis is located close to the acrosomal vacuole which results in a prolonged postcentriolar elongation of the nucleus and a small implantation fossa. This peculiar arrangement has not been reported from other Haplogynae, but is known from the entelegyne families Tetragnathidae, Araneidae and Theridiidae (Alberti, 1990; Michalik et al., in press; PM, personal observations). For example, in species of the genus *Tetragnatha* the postcentriolar elongation of the nucleus is extremely prolonged and in late spermatids it coils several times around the axoneme resulting in a cork-screw appearance (Alberti, 1990; Michalik et al., in press). A deep posterior indentation of the nucleus was also found in *P. phalangioides* and *H. pluchei*, but in these species the axonemal basis is always located at the posterior end of the nucleus (Alberti and Weinmann, 1985; Michalik et al., 2005a).

At the end of spermiogenesis, the manchette of microtubules that cover the nucleus during spermiogenesis are concentrated at the outer side of the nucleus. This is reported also from other species with an extremely prolonged postcentriolar elongation of the nucleus (Alberti, 1990; Michalik et al., in press) and may thus be a functional adaptation to the asymmetrical organization of the nucleus and the prolonged postcentriolar elongation. A flat or otherwise complex nucleus is more likely to need support during shaping than a globular nucleus. During the coiling process the manchette of microtubules disappears, which is in agreement with our assumption. The origin of the manchette of microtubules is not clear, but it seems likely that it arises from the dense material surrounding the basis of the acrosomal vacuole as reported also for other spiders (Alberti and Weinmann, 1985). Dallai

et al. (1995), working on trichopterans, described microtubules not originating from the centrioles but from clusters of dense material in the peripheral cytoplasm presumed to represent microtubule-organizing centres (MTOCs).

After the coiling process, the spermatids of *P. simoni* show a vesicular area that surrounds the axoneme and parts of the nucleus. This peculiar structure is otherwise only known from species of the haplogyne spider families Dysderidae and Oonopidae (Alberti and Weinmann, 1985; Michalik et al., 2004b). The function of the vesicular area remains unknown. It might allow a more efficient sperm activation in the female since the sperm is already provided with membranes and may thus simply "hatch" from its capsule (Michalik et al., 2004b). A similar mechanism has been suggested for the vacuolated type of sperm of several anactinotrichid mites (Oliver, 1982; Alberti and Coons, 1999). This specialization may be more widespread in haplogyne spiders than suggested by the scant data and it may not be correlated with a special type of sperm or sperm aggregation (transfer unit).

The coiled spermatids of *P. simoni* receive a secretion sheath while still in the lumen of the testis which is earlier than in any other spider species studied. Usually, the spermatozoa receive their secretion sheath within the deferent ducts (reviews by Alberti, 1990, 2000). It is noteworthy in this context that two different kinds of secretion droplets are present within the lumen of the testis of *P. simoni* whereas only one kind of droplet is present in the deferent ducts. We hypothesize that the bigger, bright secretion droplets present only in the lumen of the testis are involved in the formation of the secretion sheath. Different kinds of secretions are common in the male genital system of spiders, but it is unusual that the lumen of the testis contains more different secretions than the deferent ducts and the ejaculatory duct (Michalik and Uhl, 2005). The functions of the secretions in the seminal fluid are still unknown, but it is remarkable that the secretion droplets present in *P. simoni* are partly morphologically different from those in the seminal fluid of the other pholcid spiders studied so far. For example, two different kinds of secretion droplets were found in *H. pluchei,* one of them clearly differing in shape from those described in this study (Michalik et al., 2005a); for *P. phalangioides*, even three different kinds were described (Michalik and Uhl, 2005). The only non-morphological investigation of pholcid spider sperm so far (Uhl, 1996) used gel-electrophoretic methods and found proteinaceous substances and glyco- and lipoprotein components in the sperm storage site of female *P. phalangioides*. It is likely that the male secretions have an influence on the female as reported from several insects (e.g., Wolfner, 1997; Gillot, 2003; Chapman and Davies, 2004).

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