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 Osaki (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
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, spermio-
genesis 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: “phol-
cines” and “holocnemines” (Huber, 2000; Bruvo-Ma-
daric´, 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 postfixa-
tion in buffered 2% osmium tetroxide. After rinsing,
the tissue pieces were dehydrated in graded ethanol
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-lysine. 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 ethanol, dried in a BAL-TEC CPD 030
critical point dryer using amylacetate as intermediate,
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
nucleus and posteriorly delimited against the
nucleus by a layer of electron-dense material (Fig. 1b).
The vacuole is indented posteriorly forming a subacro-
somal 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 anterior pole of the spermatid, the two
centrioles of the axoneme are arranged in a tandem
position and migrate towards 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 indent posteriorly forming a subacros-
onal 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 plasmalem-
a is invaginated to form a flagellar tunnel (Figs. 1a, c,
d). The axoneme has a 9 x 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.
forming the so-called implantation fossa which is bordered by dense material (Figs. 1c, d). Furthermore, the nucleus 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.

**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
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–e).

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 glycoprotein 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 glycoprotein 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
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
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 opisthosoma 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).
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).
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 entelegene 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

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

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