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Ultrastructure of spermatozoa of solifuges (Arachnida, Solifugae): Possible characters for their phylogeny?

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ABSTRACT

The ultrastructure of spermatozoa is a widely accepted source of characters for phylogenetic studies. In this study the fine structure of sperm cells of representatives of six different New and Old World families (Ammotrechidae, Daesiidae, Eremobatidae, Galeodidae, Karschiidae, Solpugidae) of solifuges (Arachnida, Solifugae) were investigated in order to reveal putative characters suitable for subsequent systematic and phylogenetic analyses. The spermatozoa of solifuges represent a relatively simple type of sperm cells. In general, their spermatozoa are roundish, oval shaped (Ammotrechidae, Daesiidae, Eremobatidae, Solpugidae) or plate-shaped (Karschiidae) with or without membrane protuberances and devoid of a flagellum. Only in Galeodidae, very conspicuous thin and elongated sperm cells occur. The spermatozoa either occur as single cells (Eremobatidae, Solpugidae) or in groups of loose knit cells (Ammotrechidae) or in highly ordered groups (Karschiidae). In contrast to the other families studied here, within the Galeodidae and in the genus *Blossia* (Daesiidae) sperm cells surrounded by a secretion sheath, clearly representing coenospermia, could be observed.

1. Introduction

"The spermatozoon is a highly complicated cell and has undergone a morphological evolution which has no parallel in other cell types" (Baccetti and Afzelius, 1976). Franzén (1956) showed the intriguing variety of sperm morphology among invertebrates and correlated his findings to the mode of sperm transfer in different groups of invertebrates. In a comprehensive review, Franzén (1970) and Wirth (1984) pointed out the potential value of sperm morphology for phylogenetic studies. The use of the fine structure of spermatozoa is now widely accepted for taxonomy and phylogeny and its value has clearly been demonstrated in various groups such as, Plathelminthes, Chelicerata, Insecta, Myriapoda and Mammalia (Liana and Litvaitis, 2007; Alberti, 1991, 2000; Jamieson et al., 1999; Baccetti and Dallai, 1978; Downing Meisner et al., 2005).

Within Arachnida three different types of spermatozoa can be distinguished: the filiform-flagellate type which only occurs in Scorpiones and which is regarded as the most primitive one in arachnids, the coiled-flagellate type which is present in Pseudoscorpiones, Uropygi, Amblypygi, Araneae and Ricinulei and the aflagellate type which can be found in Opiliones, Palpigradi, Acari and Solifugae (summary in Alberti, 2000).

Solifugae are a mesodiverse order of the Arachnida and comprise 1075 species which are accommodated in 140 genera and 12 families (Harvey, 2003). The most comprehensive taxonomic treatment of the order Solifugae was done by Roewer (1934, 1941) but this has been constructively criticized by various authors (e.g., Lawrence, 1955; Muma, 1951, 1976; Harvey, 2002), due to his reliance on considerably variable characters such as spine-like leg setae on which Roewer based much of his taxon delineation. Roewer also made the first attempt to establish a phylogeny of Solifugae on family level, but to date, there is just one modern phylogenetic analysis for a species-group of the genus *Eremobates* carried out by Brookhart and Cushing (2004). Muma (1976) established a familial system based partly on characters used by Roewer, but primarily on male secondary sexual characters supported by cheliceral dentition and female opercula. Sperm morphology in Solifugae was first investigated by Alberti (1980c) and later by Alberti and Peretti (2002) and Klann et al. (2005). Although these first results show variation between the investigated representatives of the families Karschiidae, Ammotrechidae and Eremobatidae, the data on sperm ultrastructure continue to be fragmentary. The present study aims

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to investigate the morphological diversity of solifugid spermatozoa from a comparative point of view and to possibly reveal potential characters which might be applicable to the phylogeny and systematics of Solifugae.

2. Materials and methods

2.1. Animals

Summarized in Table 1.

2.2. Transmission electron microscopy (TEM)

Males were sacrificed and genital systems were removed and fixed in 2.5% glutaraldehyde buffered phosphate buffer (0.1 M, pH 7.2, 1.8% sucrose) at 4 °C overnight. Specimens were rinsed in phosphate buffer and post-fixed with aqueous OsO_4 at 4 °C for 2 h and then dehydrated in graded ethanols (60%-absolute). Finally the specimens were embedded in Spurr's medium (Spurr, 1969). Ultrathin sections of 50–70 nm were made with a diamond knife using a Leica Ultracut microtome. Sections were stained with saturated uranyl acetate and lead citrate according to the method of Reynolds (1963) and investigated with a JEOL-JEM-1011 transmission electron microscope. Glycogen detection was performed according to the method of Thiéry (1967). The sections placed on gold grids were treated with 1% periodic acid for 20 min, thoroughly rinsed with deionized water (A. deion.) and afterwards incubated with 1% thiosemicarbazide in 10% acetic acid for 24 h. Thereafter the sections were rinsed in a decreasing acetic acid series (10%, 5%, 1%) and A. deion. Finally the sections were treated with 1% silver proteinate for 30 min and rinsed with A. deion.

2.3. Scanning electron microscopy (SEM)

The male genital systems of species of the family Galeodidae were dissected, fixed and dehydrated as mentioned above. After dehydration the genital systems were transferred to amylacetate as intermedium. The specimens were critical point dried in a BAL-TEC CPD 030, sputter coated with gold–palladium (Quorum Technologies SC7620) and finally investigated with a LEO DSM 940A scanning electron microscope.

3. Results

Spermatozoa of solifuges are usually roundish or oval in shape (except for spermatozoa of Galeodidae) and consist of the chromatin body (no nuclear envelope, which is characteristic of a nucleus, could be observed) and an acrosomal complex with its acrosomal vacuole, subacrosomal material and acrosomal filament. Since no spermiogenesis could be observed, we summarized the following possible characters in solifugid spermatozoa based on mature spermatozoa as shown in Table 2: (1) aggregation in testis, (2) aggregation in vas deferens, (3) shape of sperm cell, (4) shape of chromatin body, (5) location of acrosomal complex, (6) shape of acrosomal vacuole and (7) presence or absence of glycogen granules. Differences in the ultrastructure are most obvious between representatives of different families and between certain species of the same family in the case of Daesiidae. For this reason the spermatozoa of some species are not described separately in detail. Spermiogenesis could be observed in none of the studied species here.

3.1. Ammotrechidae

In *Oltacola chacoensis* and *Nothopuga* sp. the sperm cells are organized in groups within the epithelium of the testis. The sperm cells possess finger-like curving protuberances of the membranes which interdigitate, thus forming more or less large groups (Figs. 1 and 4). It seems that these groups of spermatozoa are larger in *Nothopuga* sp. than in *O. chacoensis*. In *O. chacoensis* there are only individual sperm cells in the lumen of the vas deferens exhibiting a more flower-like shape (Fig. 3) whereas spermatozoa of *Nothopuga* sp. remain in groups in the lumen of the vas deferens (single sperm cells could only rarely be observed) (Fig. 6). In both species the chromatin body is somewhat irregularly roundish to oval shaped slightly extending into the finger-like membrane processes. In *O. chacoensis* the chromatin body is around 2–3 μm in diameter and in *Nothopuga* sp. approximately 2 μm. In both species glycogen could clearly be observed (Figs. 1, 4 and 38). The acrosomal complex is

Table 1
List of investigated species

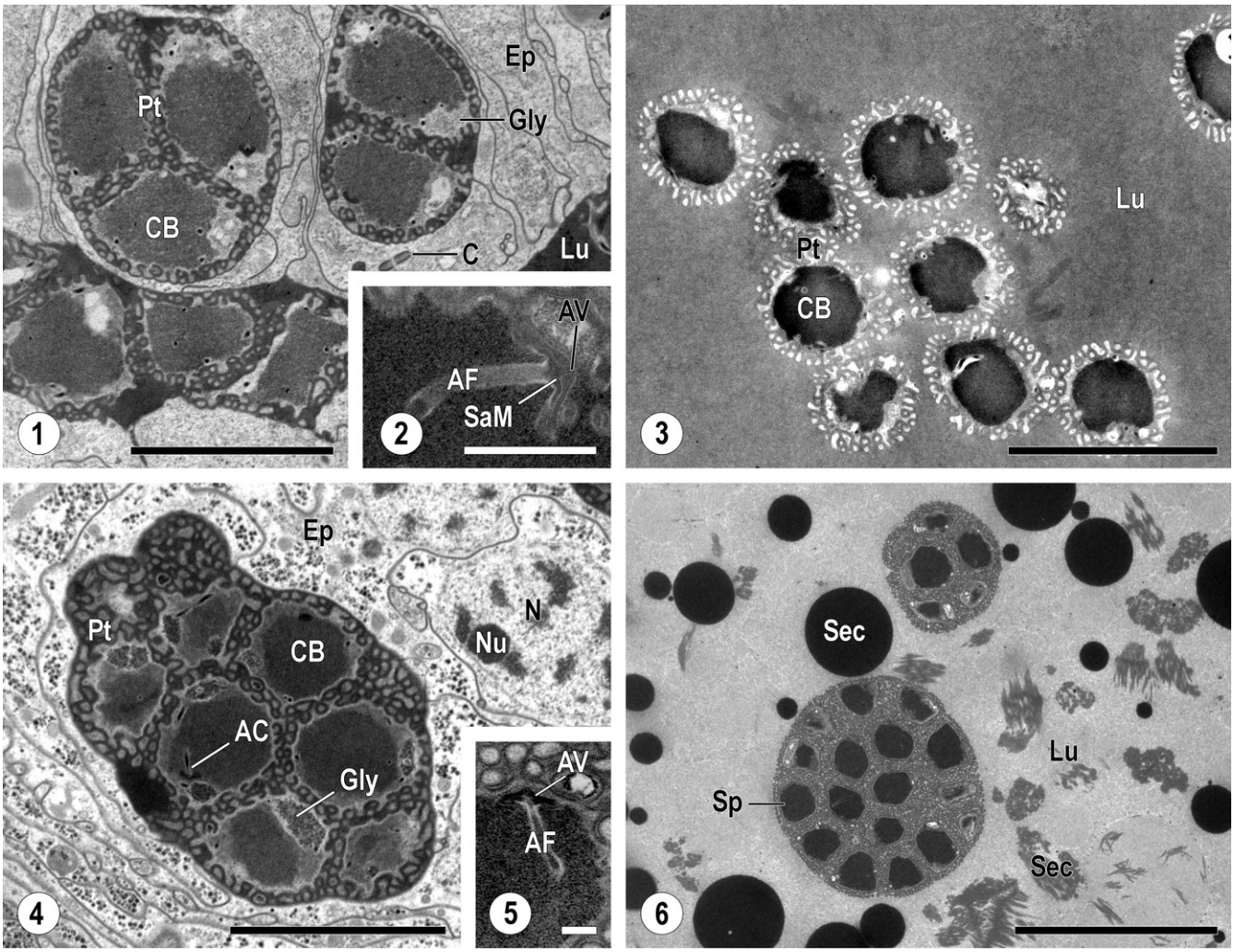
Family	Species	Country	Collecting site (GPS coordiates)	Accession No.
Ammotrechidae	*Nothopuga* sp. nov.	Argentina	64°48′23″S, 30°02′31″W, near San José de las Salinas in the Salinas Grandes, Province of Córdoba	ZIMG Acc. Cat. II 27666
	Oltacola chacoensis Roewer, 1934	Argentina	64°48′23″S, 30°02′31″W, near San José de las Salinas in the Salinas Grandes, Province of Córdoba	ZIMG Acc. Cat. II 27674
Daesiidae	*Biton striatus* (Lawrence, 1928)	Namibia	20°35′51.2″S, 14°22′08.5″E, Twyfelfontain	SMN 13504
	Biton tigrinus Pocock, 1898	Tanzania	4°47′50.2″S, 34°15′54.5″E, Mahenge, approx. 50 km west of Singida	ZIMG Acc. Cat. II 27672
	Blossia longipalpis (Lawrence, 1935)	Namibia	26°05′26.1″S, 16°13′02.2″E, at Tiras Mountains	SMN 13506
	Blossia purpurea Wharton, 1981	Namibia	25°39′13.5″S, 16°10′58.9″E, close to Tiras Mountains	SMN 13507
Eremobatidae	*Eremobates aztecus* Pocock, 1902	Mexico	San Bartolo Cuautlalpan, State of Mexico	–
	Eremobates pallipes sp. group	Mexico	20°07′21″N, 98°44′09″W, near Pachuca-City, State of Hidalgo	–
Galeodidae	*Galeodes caspius subfuscus* Birula, 1937	Kazakhstan	43°57′52.5″N, 77°03′15.9″E	ZIMG Acc. Cat. II 27667
	Galeodes turkestanus Kraepelin, 1899	Kazakhstan	42°14′23.1″N, 67°51′00.52″E	ZIMG Acc. Cat. II 27668
	Paragaleodes pallidus (Birula, 1890)	Kazakhstan	41°14′44.4″N, 68°06′33.4″E	ZIMG Acc. Cat. II 27669
Karschiidae	*Eusimonia* sp. nov.	Morocco	Between Goulimine and Sidi Ifni	ZIMG Acc. Cat. II 27671
Solpugidae	*Solpugella asiatica* Roewer, 1933	Morocco	Between Goulimine and Sidi Ifni	ZIMG Acc. Cat. II 27675
	Solpugista bicolor (Lawrence, 1953)	Namibia	23°33′21.9″S, 15°02′30.4″E, Gobabeb	SMN 13454
	Zeria venator (Pocock, 1897)	Namibia	26°57′47.8″S, 17°31′31.5″E, Vogelstrausskluft Country Lodge	SMN 13540

ZIMG = Zoologisches Institut und Museum Greifswald; SMN = State Museum Namibia.

Table 2
Distribution of character states in the sperm of different solifugid species

	Aggregation in testis	Aggregation in vas deferens	Sperm cell	Chromatin body (CB)	Location of AC[a]	AV[b]	Gly[c]
Ammotrechidae							
O. chacoensis[d]	Groups	Single	Roundish, finger-like protuberances	Roundish, slightly irregular margin	Periphery of CB	Conical	Present
Nothopuga sp.[e]	Groups	Groups	Roundish, finger-like protuberances	Roundish, slightly irregular margin	Periphery of CB	Flat conical	Present
Daesiidae							
Blossia longipalpis	Groups + thin secretion sheath	Groups + thin secretion sheath	Roundish, finger-like protuberances	Roundish/oval, irregular shape	Periphery of CB	Flat conical	Present
Blossia purpurea	Groups + thin secretion sheath	Groups + thin secretion sheath	Roundish, finger-like protuberances	Roundish/oval, slightly irregular shape	Periphery of CB	Flat conical	Present
Biton striatus	Groups	Single	Oval/roundish, finger-like protuberances	Roundish/oval, irregular shape	Periphery of CB	Flat conical	Present
Biton tigrinus	Groups	Single	Roundish, finger-like protuberances	Roundish/oval, slightly irregular shape	Within CB	Flat conical	Present
Eremobatidae							
E. aztecus	Single	Single	Roundish,flat membrane process	Oval	On CB	Discoidal	Present
E. pallipes sp. group	Single	Single	Roundish,flat membrane process	Oval	On CB	Discoidal	Present
Galeodidae							
G. turkestanus	n.o.	Groups + secretion sheath	Thread-like	Elongated	n.o.	n.o.	Absent
G. caspius subfuscus	n.o.	Groups + secretion sheath	Thread-like	Elongated	On CB	Elongated cap	Absent
P. pallidus	n.o.	Groups + secretion sheath	Thread-like	Elongated	On CB	Elongated cap	Absent
Karschiidae							
Eusimonia sp. nov.	Groups	Groups	Plate-shaped	Plate-shaped	Sunken in CB	Discoidal	Absent
Eusimonia mirabilis[f]	Groups	Groups	Plate-shaped	Plate-shaped	Sunken in CB	Discoidal	Absent
Solpugidae							
S. bicolor	Single	Single	Roundish, finger-like protuberances	Roundish	Within CB	Conical	Present
S. asiatica	Single	Single	Roundish, finger-like protuberances	Roundish	Within CB	Conical	Present
Z. venator	Single	Single	Roundish, finger-like protuberances	Roundish	Within CB	Conical	Present

n.o.: not observed.
[a] Acrosomal complex.
[b] Acrosomal vacuole.
[c] Glycogen.
[d] Data partially from Alberti and Peretti (2002): published as *Procleobis patagonicus*.
[e] Data partially from Alberti and Peretti (2002): published as *Oltacola gomezi*.
[f] Data from Alberti (1980c).

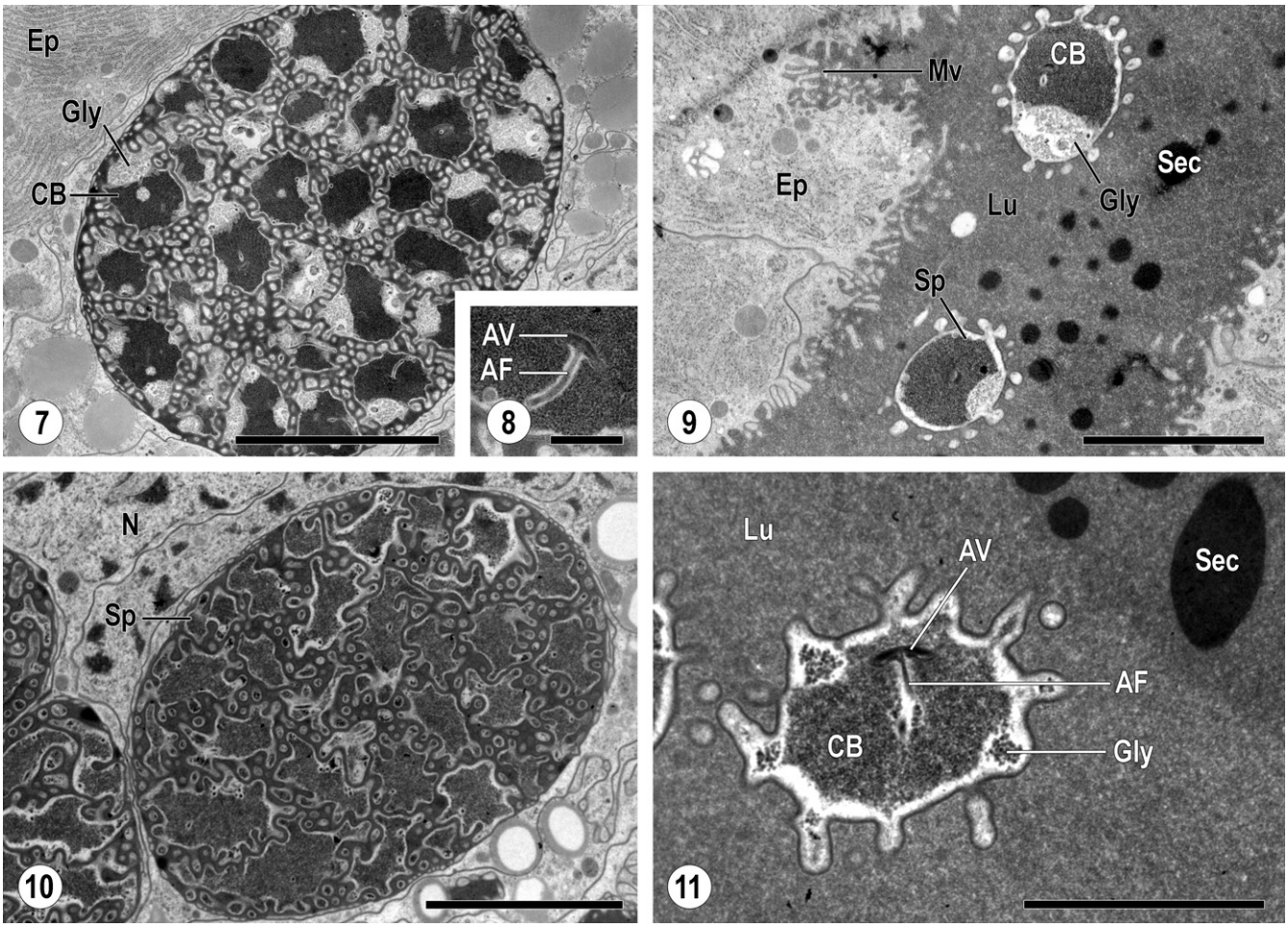


Figs. 1–6. Spermatzoa of two species of Ammotrechidae. Fig. 1. Spermatozoa of *Oltacola chacoensis* embedded in the epithelium and in the lumen of the testis. Within the epithelium they remain in groups by interdigitating membrane protuberances. TEM. Scale bar: 5 μm. Fig. 2. Conical acrosomal complex with the acrosomal vacuole and the acrosomal filament originating from the subacrosomal material. TEM. Scale bar: 500 nm. Fig. 3. Individual sperm cells with short finger-like membrane protuberances in the lumen of the vas deferens. TEM. Scale bar: 5 μm. Fig. 4. Group of spermatozoa of *Nothopuga* sp. within the epithelium. Also these sperm cells are connected via membrane protuberances to each other. TEM. Scale bar: 5 μm. Fig. 5. The acrosomal complex is flat conical and its filament penetrates the chromatin body. TEM. Scale bar: 250 nm. Fig. 6. In contrast to the spermatozoa of *O. chacoensis* the spermatozoa of *Nothopuga* sp. remain in groups in the lumen of the vas deferens. Very conspicuous are different kinds of secretions. TEM. Scale bar: 10 μm. AC: acrosomal complex; AF: acrosomal filament; AV: acrosomal vacuole; C: centriole; CB: chromatin body; Ep: epithelium; Gly: glycogen; Lu: lumen; N: nucleus; Nu: nucleolus; Pt: protuberances; SaM: subacrosomal material; Sec: secretion; Sp: spermatozoa.

very similar in both species. It consists of a conical-shaped acrosomal vacuole (which is a little bit more tapering in *O. chacoensis* than in *Nothopuga* sp.) with the subacrosomal material projecting into it (Figs. 2 and 5). The acrosomal filament arising from the subacrosomal material not only penetrates the chromatin body but it also coils around it. In both species the acrosomal complex is located more towards the chromatin body periphery.

3.2. Daesiidae

In all investigated species, the spermatozoa occur in groups within the epithelium of the testis (Figs. 7, 10, 12 and 15). In contrast to the investigated species of the genus *Blossia*, the spermatozoa of *Biton* occur individually in the vas deferens (Figs. 9 and 11). In *Biton* it seems that the spermatozoa with their curving membrane protuberances are embedded within a secretion in the epithelium of the testis (Figs. 7 and 10). The spermatozoa of *Blossia* also exhibit a meshwork of curving membranes, which seem to be surrounded by a thin secretion sheath (Figs. 12, 14, 15, 17, 40 and 41). This secretion sheath exhibits a slight texture in *Blossia purpurea*. The shape of the chromatin body in all species of this family investigated in this study is irregular with short, stout finger-like protuberances, which are of different lengths in the different species. In *Biton striatus* the diameter of the chromatin body ranges from 2 μm to 4 μm whereas in *Biton tigrinus* the diameter is less than 2 μm. The average diameter of the chromatin bodies of sperm cells of *Blossia longipalpis* and *Blossia purpurea* is around 2 μm. In *Blossia longipalpis* the chromatin bodies exhibit small areas with glycogen (Figs. 12, 14 and 40). In *Biton striatus* and *Blossia pupurea* glycogen could often be observed in groups closely located to the chromatin body (Figs. 39 and 41). The acrosomal complex is very similar in all studied daesiid species, namely flat conical-shaped (Figs. 8, 11, 13 and 16) and located towards the periphery of the chromatin body, except for *Biton tigrinus*, where the acrosomal complex is located within the chromatin body.



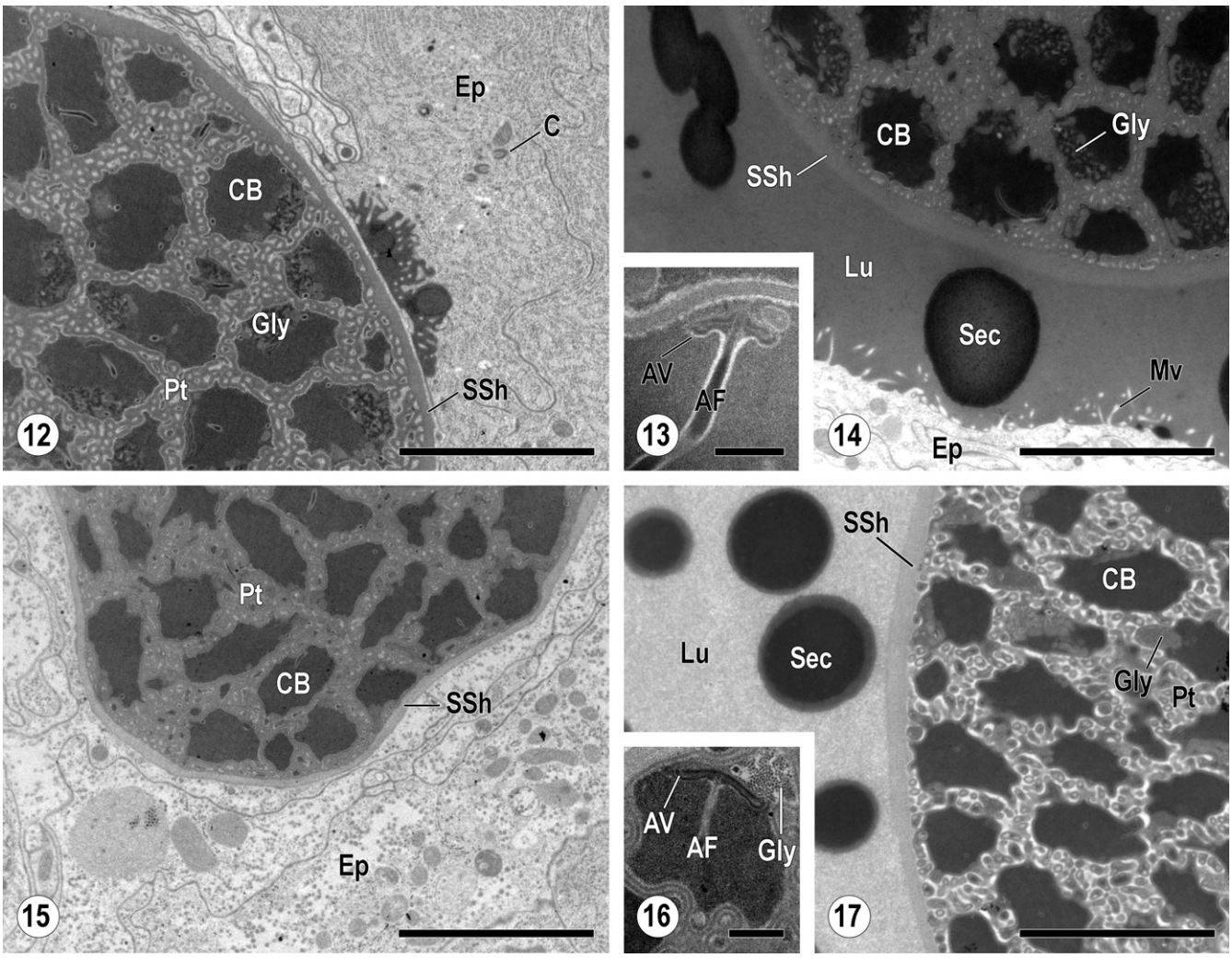
Figs. 7–11. Sperm cells of representatives of the genus *Biton* (Daesiidae). Fig. 7. Group of mature spermatozoa of *Biton tigrinus*. The individual cells possess slightly irregularly-shaped chromatin bodies and finger-like protuberances. These sperms seem to be embedded in a secretion. TEM. Scale bar: 5 μm. Fig. 8. The flat conical acrosomal complex is located within the chromatin body. TEM. Scale bar: 500 nm. Fig. 9. In the lumen of the testis the spermatozoa do not form groups any longer. TEM. Scale bar: 3 μm. Fig. 10. The sperm cells of *Biton striatus* resemble the sperm cells of *Biton tigrinus* but exhibit a much more irregular shape. TEM. Scale bar: 5 μm. Fig. 11. In the lumen of the vas deferens the spermatozoa occur individually. The small acrosomal complex is located within the chromatin body towards its periphery. Very obvious are the small glycogen granules grouped around the chromatin body. TEM. Scale bar: 2 μm. AF: acrosomal filament; AV: acrosomal vacuole; CB: chromatin body; Ep: epithelium; Gly: glycogen; Lu: lumen; Mv: microvilli; N: nucleus; Pt: protuberances; Sec: secretion; Sp: spermatozoa.

3.3. Eremobatidae

Spermatozoa of *E. aztecus* and of the male of *Eremobates* sp. are extremely similar with almost no significant differences. In both species the spermatozoa are distributed as individual cells in the epithelium of the testis and in the vas deferens as well (Figs. 18, 19, 21 and 22). Their shape is oval and they exhibit only a flat membrane extension which folds onto the cell body (Figs. 19 and 22). The chromatin body is also oval shaped and penetrated by the acrosomal filament originating from the subacrosomal material underneath the flat, discoidal acrosomal vacuole, which is in both species located on the chromatin body (Figs. 20 and 22). The spermatozoa of both species contain glycogen (Figs. 19, 22 and 42). The diameter of the chromatin body of *E. aztecus* is approximately 2 μm and in the representative of *Eremobates* sp. ranges between 1.6 μm and 2.4 μm. After leaving the chromatin body the acrosomal filament coils approximately 1.5 times around it (Figs. 20 and 22). In *E. aztecus* multilamellar bodies could be observed within the spermatozoa (Figs. 19 and 42).

3.4. Galeodidae

In contrast to all the other investigated families in this study, the spermatozoa of the genera *Galeodes* and *Paragaleodes* (Galeodidae) are encapsulated with a thick secretion sheath within the vas deferens forming oval aggregates (Figs. 23 and 24). Numerous single, extremely elongated spermatozoa cluster and form oval-shaped sub-groups. These sub-groups aggregate and are surrounded by a secretion sheath forming a highly complex coenospermium (Fig. 25). Interestingly, in all of the galeodid species studied here, no spermatozoa could be observed in the testis. The spermatozoa of *G. caspius subfuscus* and *P. pallidus* are only approximately 500 nm in diameter and the chromatin body around 250 nm (Figs. 26 and 27). The chromatin body in the investigated galeodid species is oval in cross section but it slightly tapers towards the middle and toward its ends, thus exhibiting a spindle-like shape. The chromatin itself is fibril-like structured. The acrosomal complex is situated on top of the long side of the chromatin body. The elongated cap-like acrosomal vacuole appears to be located on the chromatin body and the acrosomal filament penetrates the chromatin body. On the other



Figs. 12–17. Spermatozoa of two species of the genus *Blossia* (Daesiidae). Fig. 12. The spermatozoa of *Blossia longipalpis* exhibit roundish to oval chromatin bodies and rather long finger-like membrane protuberances forming a meshwork. Already in the epithelium of the testis the groups are surrounded by a distinct secretion sheath. In the periphery of the chromatin body of each sperm cell glycogen granules can be seen. TEM. Scale bar: 4 μm. Fig. 13. The acrosomal complex is rather small, conical and located towards the periphery of the chromatin body. Its filament penetrates the chromatin body. TEM. Scale bar: 400 nm. Fig. 14. In the lumen of the vas deferens the spermatozoa remain in groups. TEM. Scale bar: 4 μm. Fig. 15. The chromatin bodies of the spermatozoa of *Blossia purpurea* are roundish and irregularly-shaped. The groups of sperm cells are also surrounded by a secretion sheath. TEM. Scale bar: 4 μm. Fig. 16. The acrosomal vacuole is relatively flat conical-shaped and located towards the periphery of the chromatin body. Glycogen granules are clearly visible. TEM. Scale bar: 500 nm. Fig. 17. Groups of mature spermatozoa within the lumen of the vas deferens. TEM. Scale bar: 4 μm. AF: acrosomal filament; AV: acrosomal vacuole; C: centriole; CB: chromatin body; Ep: epithelium; Gly: glycogen; Lu: lumen; Mv: microvilli; Pt: protuberances; Sec: secretion; SSh: secretion sheath.

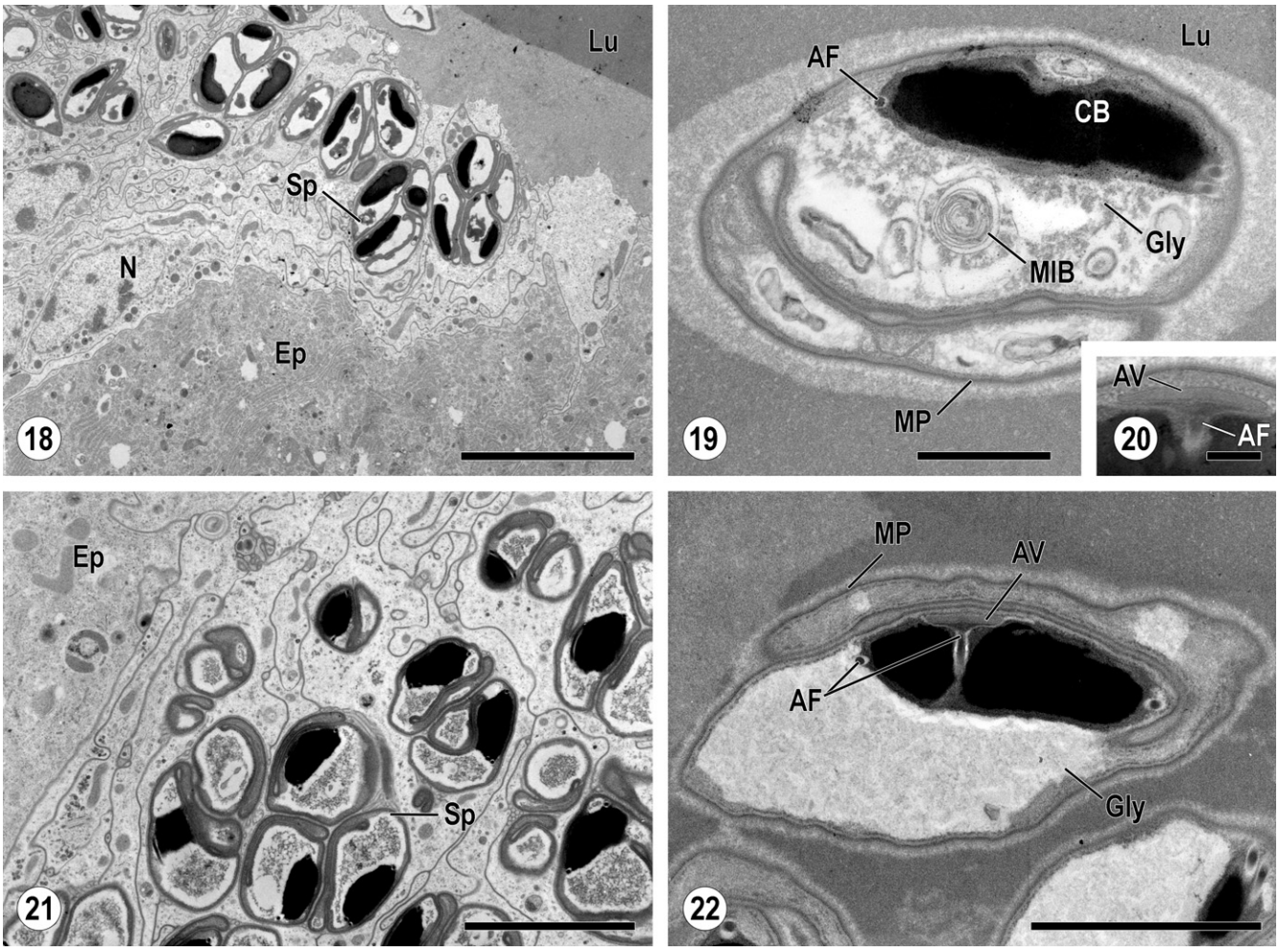
side of the chromatin body the acrosomal filament runs proximally parallel to it in a furrow and further distally free (Figs. 26–28).

3.5. Karschiidae

In the genus *Eusimonia* the spermatozoa are highly ordered in groups, in a more sophisticated way than in other investigated families. Different numbers of mature spermatozoa form stacks which can be observed in the epithelium of the testis and in the lumen of the vas deferens (Figs. 29 and 30). These groups are not surrounded by secretion sheaths. The sperm cells themselves are plate-shaped. The chromatin body is around 3 μm in diameter and approximately 80 nm high. Within these stacks of spermatozoa the acrosomal complexes of two adjacent sperm cells are always orientated towards each other. The discoidal acrosomal vacuole is sunken into the chromatin body which is penetrated by the acrosomal filament coiling more or less twice under the chromatin body in a defined area, so that in transverse sections the acrosomal filaments appear next to each other (Figs. 30 and 31).

3.6. Solpugidae

Spermatozoa of this family are relatively simple and generally do not form any type of ordered group. Sometimes they still cling together within the epithelium of the testis (Figs. 32, 34 and 36). Mature sperm cells are roundish with several finger-like processes of the cell membrane either on one or two sides and possess glycogen (Figs. 33, 35, 37 and 43). In *Solpugista bicolor* the chromatin body is approximately 1.5–2 μm in diameter, in *Zeria venator* about 2 μm and in *Solpugella asiatica* around 1.5 μm. Interestingly, the conical-shaped acrosomal complex has no defined position in the cells in none of the investigated solpugid species. It is rather situated within the chromatin body which exhibits a fibril-like structure. The acrosomal vacuole is relatively small and conical. The acrosomal filament runs through the chromatin body and coils around it (Figs. 33, 35 and 37).



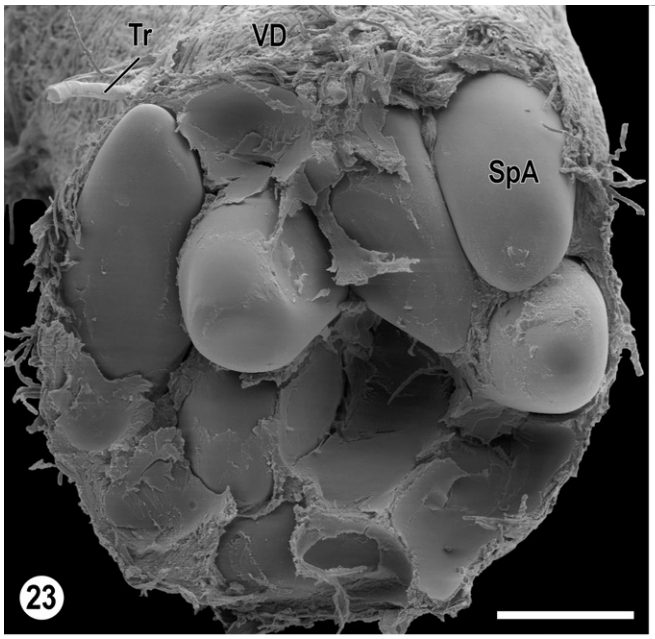
Figs. 18–22. Sperm cells of the genus *Eremobates* (Eremobatidae). Fig. 18. Spermatozoa of *E. aztecus* within the epithelium of the testis. The spermatozoa do not show a tendency to form well-ordered groups; they are only randomly aggregated. They are oval in shape. TEM. Scale bar: 10 μm. Fig. 19. The sperm cell exhibits a single flat membrane process folded onto the cell. Inside the cell, multilamellar bodies can be observed. TEM. Scale bar: 1 μm. Fig. 20. The flat, discoidal acrosomal vacuole is located on top of the chromatin body. TEM. Scale bar: 250 nm. Fig. 21. The spermatozoa of the male *Eremobates* sp. are also loosely aggregated. The oval cells possess membrane processes differently folded onto the cell body. TEM. Scale bar: 4 μm. Fig. 22. In the lumen of the vas deferens, the spermatozoa do not aggregate. The flat, discoidal acrosomal vacuole is located on top of the chromatin body. The acrosomal filament penetrates the chromatin body and coils around it. TEM. Scale bar: 2 μm. AF: acrosomal filament; AV: acrosomal vacuole; CB: chromatin body; Ep: epithelium; Gly: glycogen; Lu: lumen; MlB: multilamellar body; MP: membrane process; N: nucleus; Sp: spermatozoa.

4. Discussion

The present study shows for the first time the ultrastructure of solifugid spermatozoa from a comparative point of view. In none of the adult males of the investigated solifugid species here, stages of spermiogenesis could be detected, thus confirming former results (Alberti, 1980c; Alberti and Peretti, 2002) and supporting the hypothesis that spermiogenesis only occurs in subadult males (Klann et al., 2005) or leading to a new hypothesis, that spermiogenesis occurs shortly before or after the adult moult. In Galeodidae the production of the secretion sheath and the formation of the coenospermia probably start in the testis and end in the vas deferens. This might be the reason, why no sperm cells could be detected in the testis.

Morphological differences in the fine structure of spermatozoa in Solifugae are most obvious on family level, but nevertheless slight differences could also be observed between distinct genera of the same family, especially in the family Daesiidae. But this variation is not as considerable as it is the case in certain spider families, as e.g., in Tetragnathidae and Pholcidae (Michalik et al., 2006; Michalik and Huber, 2006). In insects and spiders, for example, spermatozoa provide a variety of characters for phylogenetic studies. Due to their flagellate character, structures such as axonemal structures, centrioles and centriole-associated structures (e.g., centriolar adjunct, implantation fossa) can be taken into consideration when analyzing sperm morphology for phylogeny (e.g., Alberti, 1990; Dallai and Afzelius, 1993; Dallai et al., 2003; Michalik, 2007). As already mentioned, the sperm cells in Solifugae are rather simple, but nevertheless different character states as shown in Table 2 could be defined. Our results already indicate that not only the presence or absence of glycogen, but also the distribution pattern of glycogen within the spermatozoa might be a character. This needs to be confirmed in future studies with additional material of those families, where glycogen could be detected in spermatozoa.

The shapes of solifugid spermatozoa are strikingly specific on family level, although slight differences could also be observed on genus level. Family level-specific differences in sperm morphology have also been reported for mites, spiders and scorpions (Alberti, 1980a,b; Alberti and Weinmann, 1985; Jespersen and Hartwick, 1973). Roewer (1934) already mentioned exomorphological similarities of the families Ammotrechidae and Daesiidae but nevertheless excluded a closer relationship between these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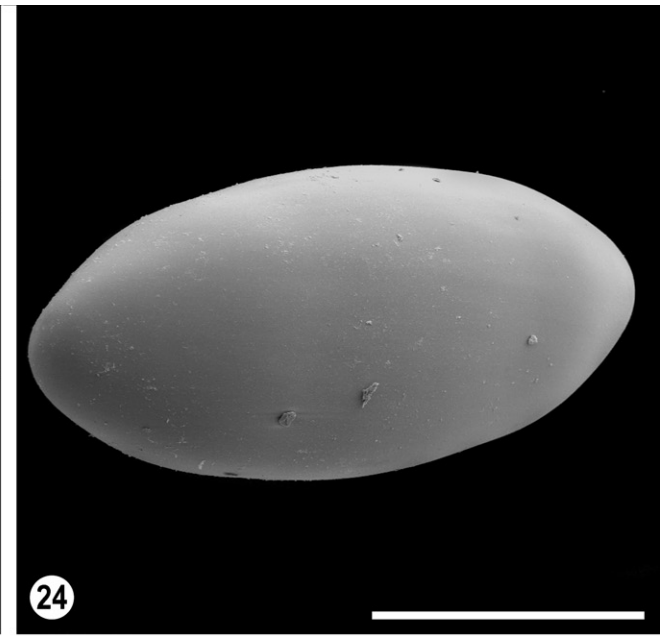
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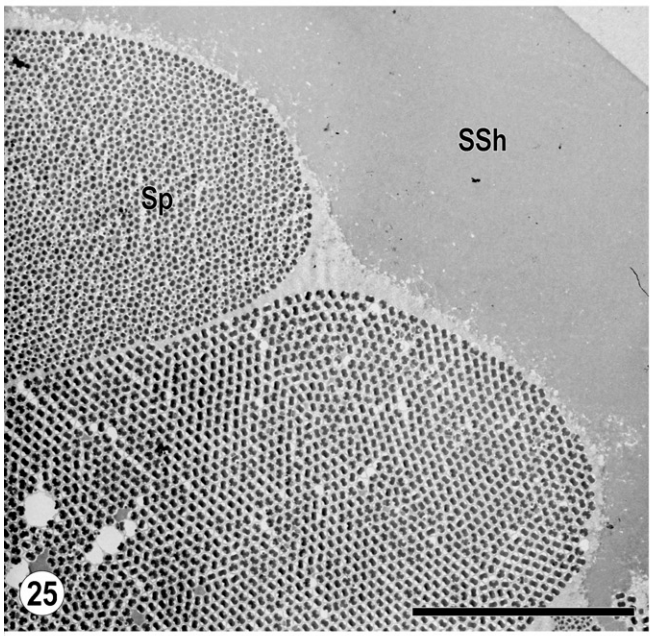
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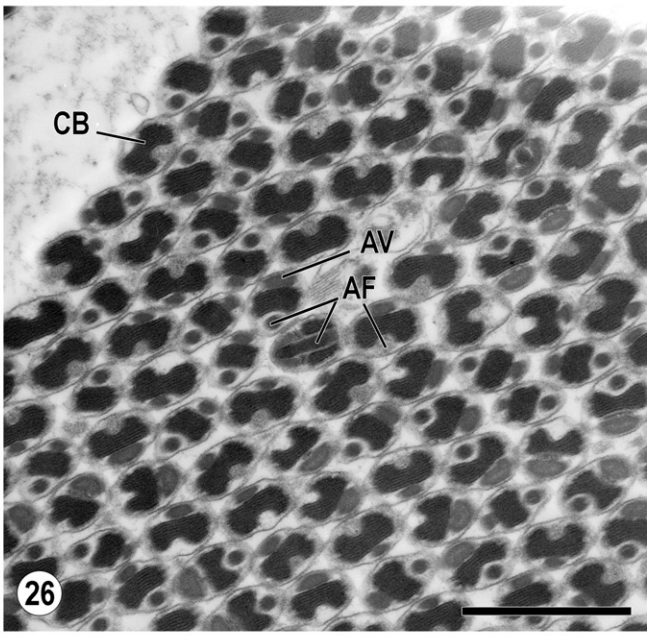
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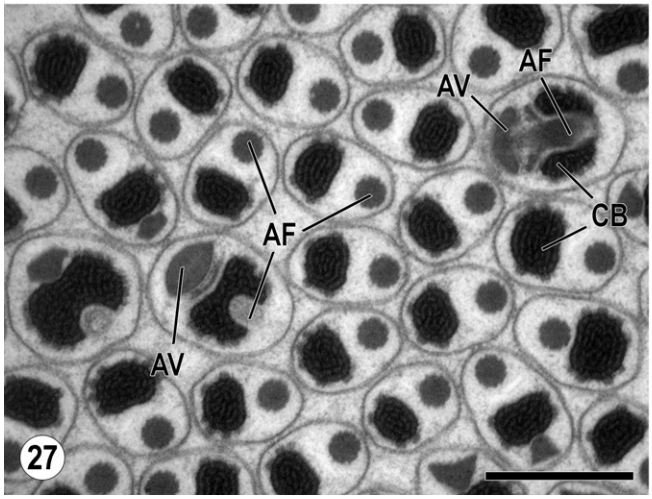
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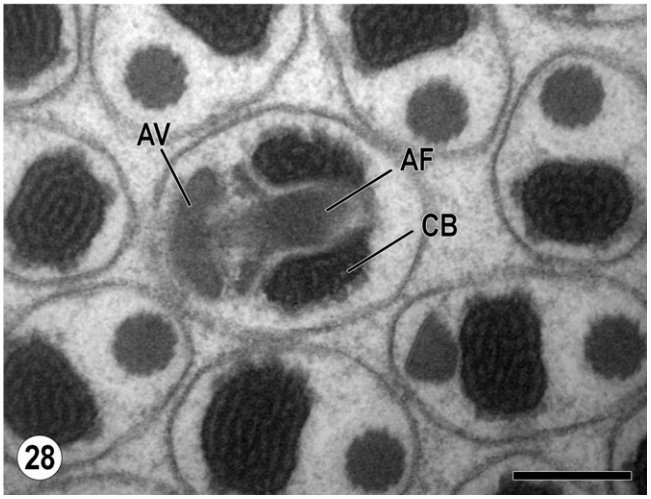
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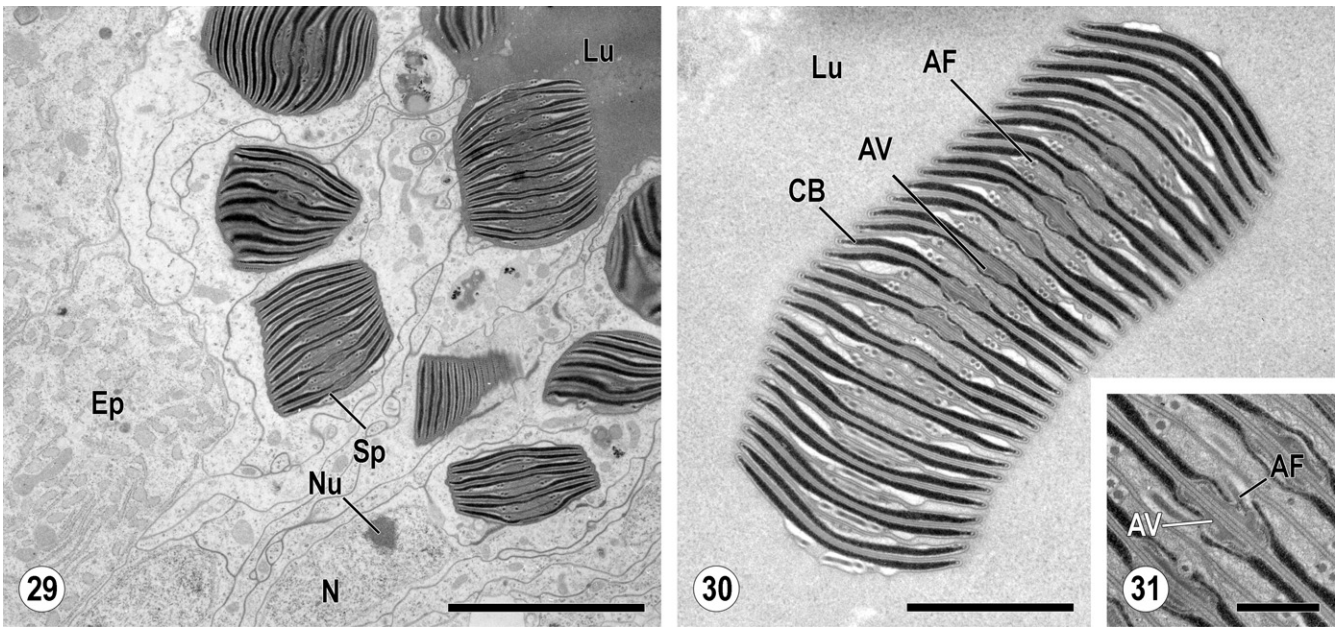


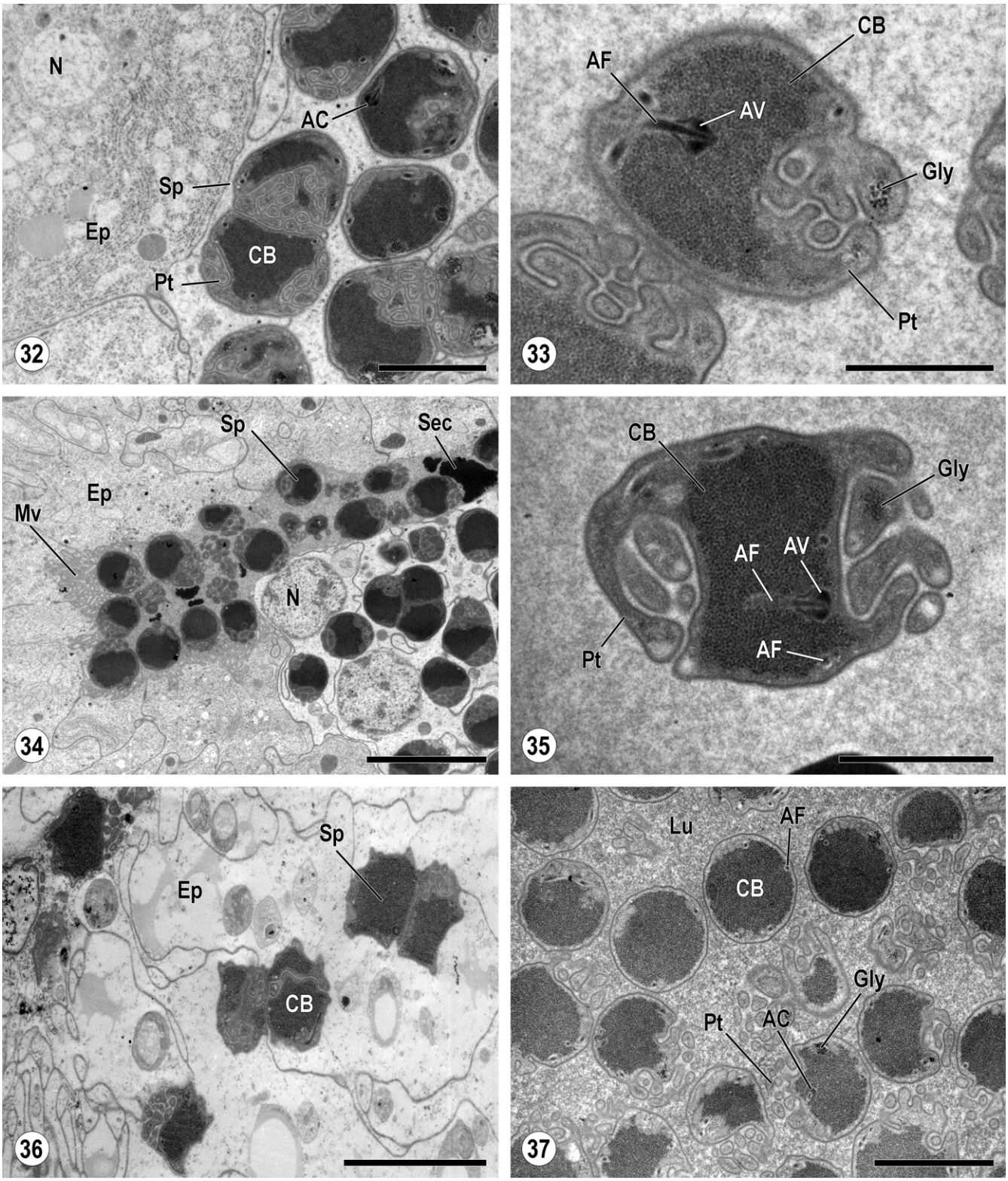
Figs. 29–31. Sperm cells of a new species of *Eusimonia* (Karschiidae). Fig. 29. Stacks of plate-shaped spermatozoa in the epithelium and the lumen of the testis of *Eusimonia* sp. nov. TEM. Scale bar: 5 μm. Fig. 30. Group of spermatozoa in the lumen of the vas deferens. Acrosomal complexes of two spermatozoa are always orientated towards each other. TEM. Scale bar: 2 μm. Fig. 31. The discoidal acrosomal complex is sunken in the chromatin body. The acrosomal filament penetrates the thin plate-shaped chromatin body and coils under it. TEM. Scale bar: 500 nm. AF: acrosomal filament; AV: acrosomal vacuole; CB: chromatin body; Ep: epithelium; Lu: lumen; N: nucleus; Nu: nucleolus; Sp: spermatozoa.

two families. Although his phylogeny of Solifugae is very controversially discussed and mostly rejected, the exomorphological similarities of Ammotrechidae and Daesiidae are also reflected in their sperm morphology. Representatives of the family Galeodidae show relatively unique morphological features of the spermatozoa among Solifugae. Not only because of their peculiar elongated form, but also because they are surrounded by a distinct, very thick secretion sheath, secreted by the epithelium of the testis and presumably the vas deferens. This type can clearly be considered as a true coenospermium. This was already observed by Kaestner (1933), but he named the encysted spermatozoa "spermatophores". Since coenospermia are defined as sperm groups surrounded by a secretion sheath within the testis or deferent duct, which occur, e.g., in different spider families such as Theraphosidae, Filistatidae and Heptathelidae (Bertkau, 1877; Alberti and Weinmann, 1985; Michalik et al., 2004) and spermatophores involve secretions of accessory glands (Alberti and Michalik, 2004), we consider the term "coenospermia" most appropriate for the galeodid sperm aggregation. Also both species of *Blossia* exhibit a thin secretion sheath and can thus also be considered as coenospermia.

Our studies again show that the highly derived sperm morphology (e.g., aflagellate, shape of the acrosomal complex, coiling of the acrosomal filament in the sperm cell) of Solifugae is most similar to those of actinotrichid mites and differs profoundly from those of Pseudoscorpiones (see also Alberti, 1980c; Alberti and Peretti, 2002). Unfortunately, these findings were incorrectly referred to by Shultz (2007), expanding these similarities to all Acari (including Anactinotrichida). But it has been demonstrated and summarized, that Actinotrichida and Anactinotrichida exhibit both exo- and endomorphological characteristics, which are very specific for each group (Alberti, 2005). Also the apomorphic testis histology (dimorphic epithelium consisting of a glandular and a germinal part) of Solifugae resembles that one of actinotrichid mites (Alberti, 1980c). But the clade Haplocnemata (Solifugae + Pseudoscorpiones), supported by synapomorphies like, e.g., the two-segmented chelicerae and their articulations, the rostrosoma and the spiracles, which was recognized by several authors in the past (e.g., Weygoldt and Paulus, 1979; Wheeler and Hayashi, 1998; Giribet et al., 2002), was recently recovered in new analyses (Shultz, 2007).

Alberti (2002) demonstrated in Gamasida (Anactinotrichida, Acari) that sperm morphology is not only correlated with the morphology of genital systems but also very likely with the mode of sperm transfer. The only systematically conducted experiment on copulatory behavior of Solifugae was done by Peretti and Willemart (2007) using *O. chacoensis* (Ammotrechidae) as a model organism. The other studies comprise descriptive observations of the mating behavior of different solifugid families in the field (Heymons, 1902; Amitai et al., 1962; Muma, 1966; Wharton, 1987) or in the laboratory (Junqua, 1966). E.g., Heymons (1902) reported that males live only for a short time after mating. In a male of *S. asiatica*

Figs. 23–28. Spermatozoa of different species of Galeodidae. Fig. 23. Scanning electron micrograph of the vas deferens of *Galeodes turkestanus*. The oval sperm aggregates clearly stick out of the lumen. Scale bar: 300 μm. Fig. 24. Scanning electron micrograph of a single coenospermium of *Galeodes caspius subfuscus*. The surface is smooth without any texture. Scale bar: 300 μm. Fig. 25. Transverse section through a coenospermium. The spermatozoa form groups, which in turn are surrounded by a relatively thick secretion sheath. TEM. Scale bar: 8 μm. Fig. 26. Transverse sections through mature spermatozoa of *G. caspius subfuscus* inside the lumen of an ovary. The acrosomal vacuole is slightly elongated, running parallel to the chromatin body. The acrosomal filament penetrates the chromatin body and runs proximally parallel in a furrow of the chromatin body. TEM. Scale bar: 1 μm. Fig. 27. Transverse sections through the spermatozoa of *Paragaleodes pallidus*. The acrosomal vacuole is located in top of the chromatin body. The acrosomal filament penetrating the chromatin body runs proximally in a furrow very similar to the spermatozoa of *G. caspius subfuscus*. TEM. Scale bar: 500 nm. Fig. 28. The acrosomal complex appears mushroom shaped. The chromatin is slightly structured. TEM. Scale bar: 200 nm. AF: acrosomal filament, AV: acrosomal vacuole, CB: chromatin body, SpA: sperm aggregate, SSh: secretion sheath, Tr: trachea, VD: vas deferens.



Figs. 32–37. Spermatozoa of three species of the family Solpugidae. Fig. 32. Spermatozoa in the epithelium of the testis of *Solpugista bicolor*. TEM. Scale bar: 2 μm. Fig. 33. Spermatozoon in the lumen of the vas deferens. Clearly visible are the finger-like membrane processes. The acrosomal complex seems to be located within the chromatin body. The acrosomal vacuole seems to be connected to the acrosomal filament via thin "processes" running from the margin of the vacuole to the filament. The acrosomal filament is coiled around the chromatin body. Glycogen granules can be observed in the finger-like processes. TEM. Scale bar: 1 μm. Fig. 34. Sperm cells in both the epithelium and the lumen of the testis of *Zeria venator*. Finger-like membrane processes can be observed on either one or two sides of the sperm cell. TEM. Scale bar: 5 μm. Fig. 35. Sperm cell in the lumen of the vas deferens. The acrosomal complex exhibits basically the same shape like that one in *S. bicolor*, only the acrosomal vacuole seems to be slightly more flat. Glycogen is also present in the finger-like processes. TEM. Scale bar: 1 μm. Fig. 36. Spermatozoa in the epithelium of the testis of *Solpugella asiatica*. Only a few spermatozoa could be observed in the male suggesting that it already had mated before dissection. Characteristic finger-like membrane protuberances could also be observed here. TEM. Scale bar: 3 μm. Fig. 37. In the lumen of the vas deferens the spermatozoa occur individually. The chromatin body is roundish, glycogen granules can be observed in groups around the chromatin body. TEM. Scale bar: 2 μm. AC: acrosomal complex; AF: acrosomal filament; AV: acrosomal vacuole; CB: chromatin body; Ep: epithelium; Gly: glycogen; Lu: lumen; Mv: microvilli; Pt: protuberances; Sec: secretion; Sp: spermatozoa.

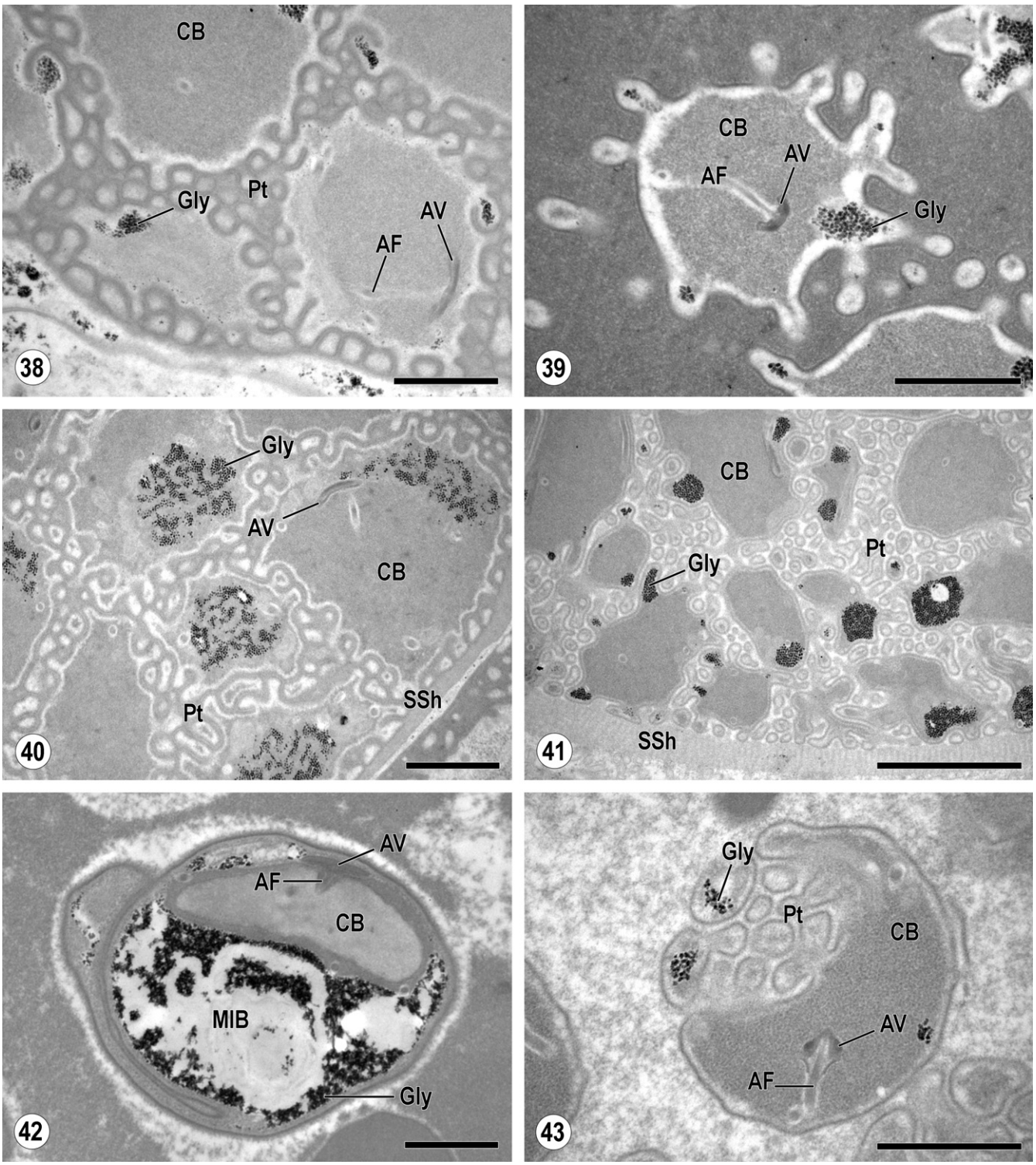


Fig. 38–43. Comparative overview of glycogen distribution patterns detected in spermatozoa of different solifuges. Fig. 38. *Nothopuga* sp. (Ammotrechidae). Glycogen is mainly aggregated in small groups closely located to the chromatin body. Scale bar: 1 μm. Fig. 39. *Biton striatus* (Daesiidae). Groups of glycogen granules, located around the chromatin body, sometimes extend into the finger-like protuberances. Scale bar: 1 μm. Fig. 40. *Blossia longipalpis* (Daesiidae). Glycogen is located between irregularly condensed chromatin forming conspicuous areas within the chromatin body. Scale bar: 1 μm. Fig. 41. *Blossia purpurea* (Daesiidae). The glycogen forms rather dense groups located closely to the chromatin body. These groups also extend into the areas of the finger-like protuberances. Scale bar: 2 μm. Fig. 42. *E. aztecus* (Eremobatidae). The glycogen is located underneath the chromatin body surrounding the multilamellar bodies. Fig. 43. *S. bicolor* (Solpugidae). The glycogen is mainly distributed within the finger-like protuberances of the sperm cell, but small groups of glycogen may also occur closely located to the chromatin body. Scale bar: 1 μm. AF: acrosomal filament, AV: acrosomal vacuole; CB: chromatin body; Gly: glycogen; MIB: multilamellar body; Pt: protuberances; SSh: secretion sheath.

conspicuously few spermatozoa could be observed in the genital system, suggesting that the testis and vasa deferentia serve only as storage sites of the spermatozoa until sperm transfer during copulation (Klann et al., 2005) and that previous mating led to sperm depletion. In contrast to Heymons (1902) and Amitai et al. (1962), who reported on amorphous sperm mass in Galeodidae, Junqua (1966) mentioned that the sperm mass consists of "numerous spermatophores in a secretion" and Hruškova-Martišková et al. (2007) showed photographs of the sperm mass for the first time. Since all authors reported that the sperm transfer from the male to the female does not take more than a few seconds, it is very likely, that Heymons (1902) and Amitai et al. (1962) were not able to thoroughly observe the sperm mass. Unfortunately, to date the morphology of the spermatozoa cannot be related to the mode of sperm transfer in solifuges, yet. Further investigations on the fine structure of spermatozoa could contribute valuably to future studies of functional aspects of sperm transfer and sperm storage in the female also in terms of fertilization and sperm competition.

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