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Comparative spermatology of freshwater mites (Hydrachnidia, Acari)

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Abstract

The ultrastructure of sperm cells of representatives of all superfamilies of Hydrachnidia except Stygothrombioidea is described. The sperm are aflagellate cells with magnitudes reaching 1.3 µm up to 6 µm. They are mostly oval cells, but some show an irregular shape. All investigated mites have an acrosomal complex which is composed of an acrosomal vacuole alone. An acrosomal filament is absent. This character together with a prominent field of granules, likely glycogen, may be regarded as synapomorphic of the groups forming a monophylum Hydrachnidia. All Hydrachnidia except *Hydrovolzia* and the Eylaoidea possess a rather large acrosomal vacuole to which a thin nuclear process attaches. This arrangement supports the taxon Euhydrachnidia. Further details shown in the fine structure of the sperm cells demonstrate the potential of these characters for the development of a better understanding of the systematic relationships within the Hydrachnidia. However, this needs further studies of more species, which should include Stygothrombioidea and observations of spermatogenesis.

Keywords: chromatin body, acrosomal vacuole, fine structure, systematics, ultrastructure

Zusammenfassung

Die Feinstruktur der Spermien von Vertretern aller Großgruppen der Hydrachnidia außer den Stygothrombioidea wird beschrieben. Die Spermien sind aflagellat und liegen in ihrer Größe zwischen 1,3 µm und 6 µm. Sie zeigen meist eine ovale Gestalt, jedoch gibt es auch sehr unregelmäßige Zellformen. Bei allen untersuchten Arten besteht der Akrosomkomplex nur aus der Akrosomvakuole, ein Akrosomfilament fehlt. Dieses Merkmal kann zusammen mit dem Auftreten eines Granulafeldes (wahrscheinlich aus Glykogen bestehend) als Synapomorphie der das Monophylum Hydrachnidia bildenden Gruppen gewertet werden. Alle Hydrachnidia außer *Hydrovolzia* und den Eylaoidea besitzen eine relativ große Akrosomvakuole, an die ein dünner Kernfortsatz heranreicht. Dieser Merkmalskomplex kennzeichnet die Euhydrachnidia. Weitere Details in der Feinstruktur der Spermien zeigen ihr Potential für die Erarbeitung eines besseren Verständnisses der systematischen Zusammenhänge innerhalb der Hydrachnidia. Hierzu müssen jedoch Untersuchungen an weiteren Arten erfolgen, die auch die Stygothrombioidea und die Besonderheiten der Spermatogenese einbeziehen sollten.

1. Introduction

Hydrachnidia (freshwater mites) comprise more than 6000 described species in more than 40 families world-wide. Traditionally, Hydrachnidia were regarded as a polyphyletic or diphyletic group within the Parasitengona (Viets 1936, Kethley 1982, Moritz 1993). On the contrary, based on investigation of larval morphology or male genital systems, Sparing (1959) and Barr (1972) considered only Hydrovolzioidea as a possible subgroup that might have evolved independently from other terrestrial Parasitengona. The detection of genital papillae (acetabula) in *Hydrovolzia placophora*, which were always overlooked in earlier studies by Alberti & Bader (1990), left the Hydrovolzioidea already in a less separate position.

Spermatozoa represent a specialised cell type, which – because of its taxon specific fine structure – can potentially provide distinctive characters for phylogenetic-systematical studies. However, in the case of Parasitengona and Hydrachnidia, the basis of such an analysis is still rather small. Nevertheless, Alberti (1980b) could show that the polyphyly-hypothesis was not supported by spermatology. However, a distinct peculiar position of *Limnochares aquatica* (Eylaoidea) was evident. Unfortunately, this previous study could not include Hydrovolzioidea. A similar interpretation was given by Witte (1991), based on extensive and detailed studies of reproductive biology of Parasitengona (see also Witte & Döring 1999, Wohltmann et al. 2001). In the present study, sperm cells of representatives of 12 families from almost all superfamilies are described (Hydrachnoidea sensu Witte 1991; Stygothrombioidea not included). For the first time, sperm cells of the still enigmatic Hydrovolzioidea are considered. Furthermore, a representative of the marine Pontarachnidae is included. Thus, the investigations of Alberti (1980b) are considerably broadened.

The male genital system of Hydrachnidia is basically composed of paired or unpaired horse-shoe-shaped testes. These continue into paired deferent ducts and finally into a very complex unpaired ejaculatory duct, where a spermatophore is shaped (Pahnke 1974). Each testis is a simple thick tube (most species) or may show distinct pouches (e.g. *Limnochares aquatica*). In cross sections it is evident that each testis consists of a germinal part, in which spermatogenesis takes place, and a glandular part as is the case in other actinotrichid mites (Alberti 1974, 1980b). Mature sperm cells and secretions are delivered into a wide lumen, where the sperm cells are covered by a secretion sheath and are surrounded by different secretions (Barr 1972, Pahnke 1974, Alberti 1980b, Alberti & Coons 1999, Smit & Alberti subm.). As in other actinotrichid mites, spermatogenesis occurs in so-called cysts, which contain gametes in the same stage of development and are surrounded by somatic cells. In a late stage, the gametes are separated from each other and delivered into the testis lumen (Alberti 1980b). Through all stages of spermatogenesis, the gametes do not show any anlage of a flagellum. Hence, the mature sperm cells are aflagellate as in all the Acari. The nuclei of mature sperm of actinotrichid mites do not show a nuclear envelope. Thus, the nuclei are represented by a chromatin body (Alberti 1980a, b, 1991, 2000, Alberti & Coons 1999).

2. Materials and methods

Details on the species considered here are listed in Tab. 1. Specimens were fixed in 2.5 – 3.5 % buffered glutaraldehyde (phosphate buffer or cacodylate buffer pH 7.4; 0.1 M) and 2 % OsO_4-aqueous solution. After dehydration in graded ethanols they were embedded in Araldite or Spurr's resin. Semithin and ultrathin sectioning was done using glass or Diatome diamond knives (for details see Alberti & Nuzzaci 1996). After staining the ultrathin sections with uranyl acetate and lead citrate, specimens were studied with transmission electron microscopes (Zeiss EM9, EM10; JEOL-JEM-1011). Only mature sperm cells from the testis lumen were considered.

Tab. 1 Species investigated with regard to comparative spermatology (taxonomy acc. to Gerecke 1994; Davids et al. 2006)

Superfamily	Family	Species	Collector	Location
Hydrovolzioidea	Hydrovolziidae	*Hydrovolzia placophora* (Monti, 1905)	P. Martin	near Berchtesgaden
Eylaoidea	Limnocharidae	*Limnochares aquatica* (Linné, 1758)	G. Alberti	near Kiel; near Gützkow
	Eylaidae	*Eylais rimosa* Piersig, 1899	G. Alberti	near Gützkow
Hydrachnoidea	Hydrachnidae	*Hydrachna cruenta* O. F. Müller, 1776	acc. to Witte (1991)	
Hydryphantoidea	Hydryphantidae	*Acerbitas* sp.	G. Alberti	near Kiel
		Protzia eximia (Protz, 1896)	P. Martin & P. Carrera	near Kiel
	Hydrodromidae	*Hydrodroma pilosa* (Besseling, 1942)	G. Alberti	Gützkow
Lebertioidea	Sperchontidae	*Sperchon setiger* Thor, 1898	F. Ullrich & G. Alberti	near Schlitz
		Sperchonopsis verrucosa (Protz, 1896)	P. Martin & P. Carrera	near Farve
Hygrobatoidea	Pontarachnidae	*Litarachna communis* Walter, 1925	H. Smit	near Ramatuelle, France
	Limnesiidae	*Limnesia maculata* (O. F. Müller, 1776)	G. Alberti	near Kiel
	Hygrobatidae	*Hygrobates nigromaculatus* Piersig, 1898	P. Martin & P. Carrera	near Farve
	Pionidae	*Piona* cf. *longipalpis* (Krendowsky, 1884)	G. Alberti	near Kiel
	Aturidae	*Aturus fontinalis* Lundblad, 1920	P. Martin & P. Carrera	near Farve
Arrenuroidea	Arrenuridae	*Arrenurus* (*Arrenurus*) *bicuspidator* Berlese, 1885	G. Alberti	near Kiel
		Arrenurus (*Truncaturus*) *fontinalis* Viets, 1920	P. Martin & P. Carrera	near Kiel

3. Results

In the following the fine structure of the mature sperm of the investigated species are shortly described.

Hydrovolzioidea

***Hydrovolzia placophora* (Monti, 1905)** (Fig. 1a, b)

Cell shape: oval; 2.0 µm long, 1.5 µm thick (largest diameter)
Cell periphery: many small, electron-lucent tubular invaginations in the anterior part of the cell, thin secretory sheath
Cell interior:
    Nucleus: elongated, homogenous, electron-dense chromatin body forming several thin processes anteriorly; chromatin body loops within the cell (frequently cut twice)
    Acrosomal complex: indistinct, small cap; no acrosomal filament
    Mitochondria: indistinct
    Further components: large granule field, large homogenous inclusions

Eylaoidea

***Limnochares aquatica* (Linné, 1758)** (Fig. 1c)

Cell shape: spherical; largest diameter: 6 µm
Cell periphery: many small, lucent tubular invaginations; thin secretion sheath
Cell interior:
    Nucleus: chromatin body relatively small, spherical, with small light spots; small thin processes extend from its surface; broad contact with acrosomal vacuole
    Acrosomal complex: acrosomal vacuole small, comprising a flat plate; no acrosomal filament
    Mitochondria: indistinct
    Further components: large granule field, membrane-bounded inclusions of unknown significance; further membranes in central parts, sometimes forming stacks

***Eylais rimosa* Piersig, 1899** (Fig. 1d)

Cell shape: spherical to lens-like; diameter: 2.5 µm
Cell periphery: a few irregular, likely long lucent invaginations; thin secretion sheath
Cell interior:
    Nucleus: chromatin body relatively small, electron-dense; massive without lucent spots; peculiar horn-like processes towards the acrosomal vacuole
    Acrosomal complex: acrosomal vacuole small, cap-like, slightly protruding; no acrosomal filament
    Mitochondria: several small, with cristae
    Further components: granule field, membrane-bounded inclusions

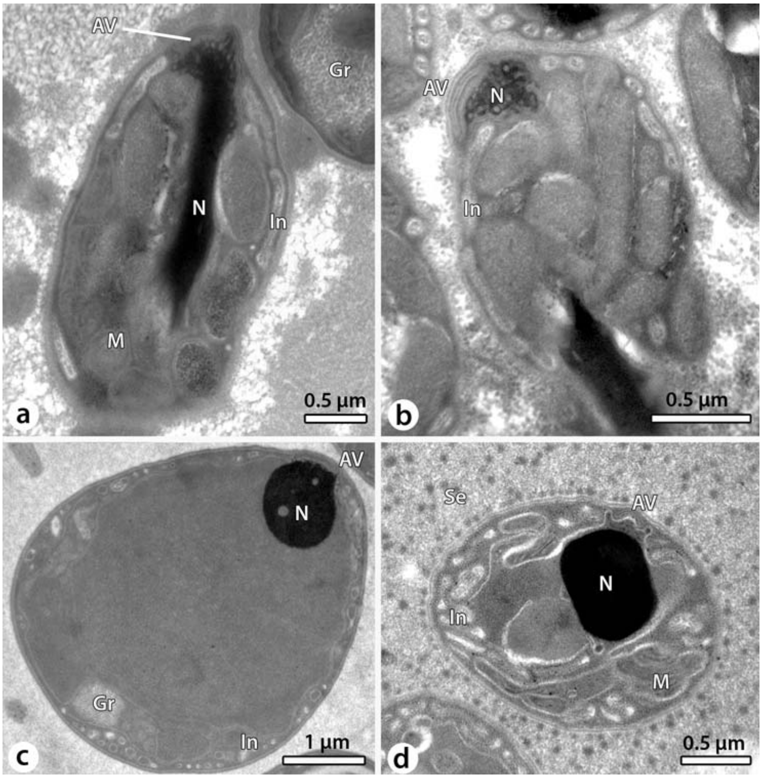

Fig. 1 a: Longitudinal section through sperm cell of *Hydrovolzia placophora* (Hydrovolziidae); b: Details of sperm cells of *H. placophora*; c: Sperm cell of *Limnochares aquatica* (Limnocharidae). Note relatively small chromatin body within large cell and cap-like acrosomal vacuole; d: Sperm cell of *Eylais rimosa* (Eylaidae). Small cap-like acrosoma vacuole. Abbr.: AV: acrosomal vacuole; Gr: field of granules; In: invaginations; M: mitochondrium; N; nucleus; Se: secretion.

Hydrachnoidea

***Hydrachna cruenta* O. F. Müller, 1776** (acc. to Witte 1991) (Fig. 2a)

Cell shape: ovoid or lens-like (?); largest diameter: about 1.5 µm
Cell periphery: many lucent, tube-like invaginations
Cell interior:
- Nucleus: unknown
- Acrosomal complex: large acrosomal vacuole; no acrosomal filament (pers. comm. R. Olomski)
- Mitochondria: unknown
- Further components: large granule field

Hydryphantoidea

Acerbitas sp. (Fig. 2b)
Cell shape: oval; 2 µm long, largest diameter: 1.5 µm
Cell periphery: many lucent, tubular invaginations; thin secretion sheath
Cell interior:

Nucleus: chromatin body relatively large, dense, without lucent parts, peripherally irregularly shaped (artifact?); thin and branching extensions towards the acrosomal vacuole

Acrosomal complex: acrosomal vacuole rather large with a cap-like protruding central part and extending sac-like peripheral parts. The latter with a distinct substructure (streaks)

Mitochondria: few, rather large, with cristae; located at the posterior pole of the cell

Further components: granule field

Protzia eximia **(Protz, 1896)** (Fig. 2c)
Cell shape: more or less spherical, but periphery often irrgular; diameter: 1.5 – 2 µm
Cell periphery: many small, lucent and tubular invaginations; thin secretion layer
Cell interior:

Nucleus: dense chromatin body with some lucent spots; massive with thin process extending to the center of acrosomal vacuole, process branches to form thin threads; some isolated chromatin granules

Acrosomal complex: acrosomal vacuole rather large with flat centre, which slightly projects; at the periphery of this centre, the vacuole widens sac-like, this part contains indistinct streaks; no acrosomal filament

Mitochondria: indistinct

Further components: large granule field, spherical inclusions (perhaps mitochondria?)

Hydrodroma pilosa **(Besseling, 1942)** (Fig. 2d)
Cell shape: almost spherical; diameter: 1.5 – 2 µm
Cell periphery: numerous lucent, tubular invaginations; thin secretion sheath
Cell interior:

Nucleus: chromatin body rather large, dense, without lucent spots; periphery irregular (artifact?); thin branching processes towards the acrosomal vacuole; some isolated chromatin granules separate from the central part of acrosomal vacuole

Acrosomal complex: quite large acrosomal vacuole set like a bell on top of the chromatin body; sac-like parts of vacuole with streaks; no acrosomal filament

Mitochondria: several, quite large with cristae; distributed irregularly within the cytoplasm

Further components: distinct granule field

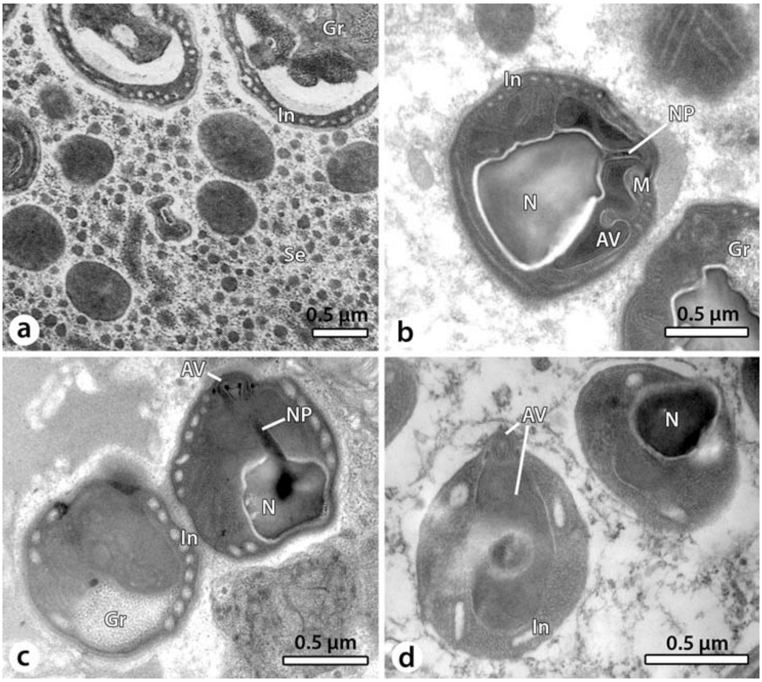

Fig. 2 a: Micrograph showing details from testis lumen of *Hydrachna cruenta* (Hydrachnidae) (modified from Witte 1991); b: Spermatozoon of *Acerbitas* sp. (Hydryphantidae). Note relatively large acrosomal vacuole; c: Two sperm cells of *Protzia eximia* (Hydryphantidae). The nuclear process is evident; d: Sperm cells of *Hydrodroma pilosa* (Hydrodromidae). Abbr.: AV, acrosomal vacuole; Gr: field of granules; In: invaginations; M: mitochondrium; N: nucleus; NP: nuclear process; Se: secretion.

Lebertioidea

***Sperchon setiger* Thor, 1898** (Fig. 3a)
Cell shape: irregular, almost bizarre; maximum length: about 4 µm
Cell periphery: tubular invaginations present, thin secretion sheath
Cell interior:
  Nucleus: chromatin body homogenous, dense, elongated, branching into thin thread-like processes towards the acrosomal vacuole
  Acrosomal complex: acrosomal vacuole with flat central part projecting slightly; lateral sac-like parts with streaks; no acrosomal filament
  Mitochondria: ovoid bodies, no cristae recognisable
  Further components: large granule field

***Sperchonopsis verrucosa* (Protz, 1896)** (Fig. 3b)

Cell shape: lengthy with irregular surface (but not as extreme as in *Sp. setiger*); maximum length: 3.5 µm

Cell periphery: long, lucent invaginations; thin secretion sheath

Cell interior:

Nucleus: homogenous, dense, massive and ovoid; with a long thin process extending under the acrosomal vacuole

Acrosomal complex: acrosomal vacuole rather large with sac-like parts containing streaks; no acrosomal filament

Mitochondria: of middle size with cristae

Further components: large granule field

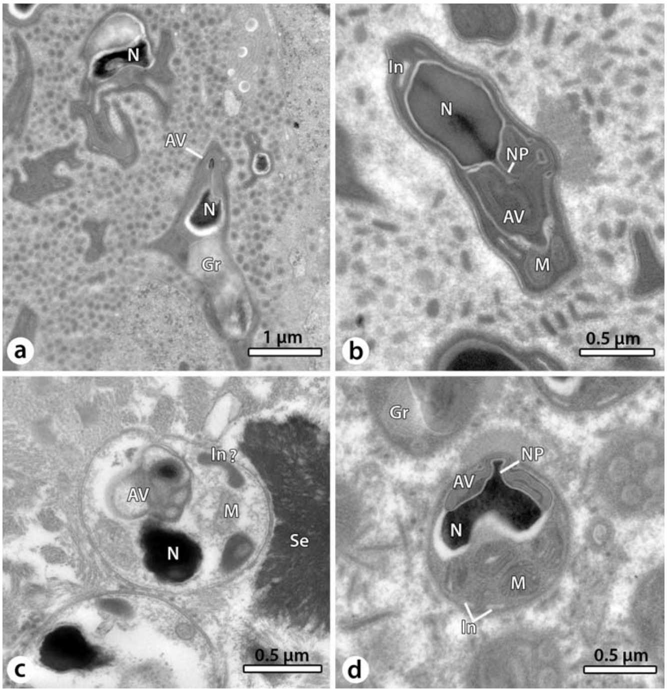

Fig. 3 a: Sperm cells of *Sperchon setiger* (Sperchontidae) show a very peculiar and complex shape; b: Spermatozoa of *Sperchonopsis verrucosa* (Sperchontidae) are elongated, but less complex in shape as *Sp. setiger*. Note large acrosomal vacuole and nuclear process; c: *Litarachna communis* (Pontarachnidae) has spherical sperm cells with a large structured and partly electron-lucent acrosomal vacuole. Note peculiar secretions within testis lumen; d: *Limnesia maculata* (Limnesiidae). The nucleus is bell-shaped and shows a process extending under the large acrosomal vacuole. Abbr.: AV: acrosomal vacuole; Gr: field of granules; In: invaginations; In?: putative invagination; M: mitochondrium; N: nucleus; N: nuclear process; Se: secretion.

Hygrobatoidea

***Litarachna communis* Walter, 1925** (Fig. 3c)

Cell shape: spherical; diameter**:** 1.4 µm

Cell periphery: without apparent peculiarities (but see below!); thin secretion sheath

Cell interior:

Nucleus: massive, slightly elongated chromatin body; nuclear process extends towards acrosomal vacuole

Acrosomal complex: rather large, structured acrosomal vacuole with lucent, fine-granular contents; no acrosomal filament

Mitochondria: few, rather large, with distinct cristae

Further components: several large dense inclusions in the periphery, which contain a lucent centre (perhaps these structures represent the tubular invaginations of other species; such invaginations are seen during spermatogenesis in *Litarachna*); a granule field was not observed

***Limnesia maculata* (O. F. Müller, 1776)** (Fig. 3d)

Cell shape: spherical; diameter: 1.3 µm

Cell periphery: few, short tubular invaginations mainly in the posterior part of the cell; thin secretion sheath

Cell interior:

Nucleus: massive chromatin body, indented from its posterior part; narrow nuclear process extends under the acrosomal vacuole; small probably isolated chromatin granules separate from the central part of acrosomal vacuole

Acrosomal complex: acrosomal vacuole composed of central slightly projecting plate and peripheral sac-like parts containing streaks; no acrosomal filament

Mitochondria: few, relatively large, located at the posterior pole of the cell

Further components: small granule field

***Hygrobates nigromaculatus* Piersig, 1898** (Fig. 4a)

Cell shape: nearly spherical; diameter: 1.5 µm

Cell periphery: thick, indistinct secretion sheath (a few deep invaginations were only seen during spermatogenesis)

Cell interior:

Nucleus: rather large, very dense, irregularly shaped (or sausage-shaped)

Acrosomal complex: large structured acrosomal vacuole; no acrosomal filament

Mitochondria: indistinct

Further components: granule field, peripheral inclusions (perhaps derived from invaginations?)

***Piona* cf. *longipalpis* (Krendowsky, 1884)** (Fig. 4b)

Cell shape: spherical; diameter: about 2 µm

Cell periphery: many lucent, tubular invaginations; indistinct secretion sheath; sperm cells not so abundant, embedded in large bodies of secretion (perhaps 'spermatophores'?)

Cell interior:

Nucleus: chromatin body elongated with irregular periphery, looping through the cell; nuclear process extends under the acrosomal vacuole

Acrosomal complex: cap-like, peripherally enlarged as a flat (not sac-like) structure, sunken into the cell (not touching the periphery); no acrosomal filament

Mitochondria: indistinct

Further components: large granule field

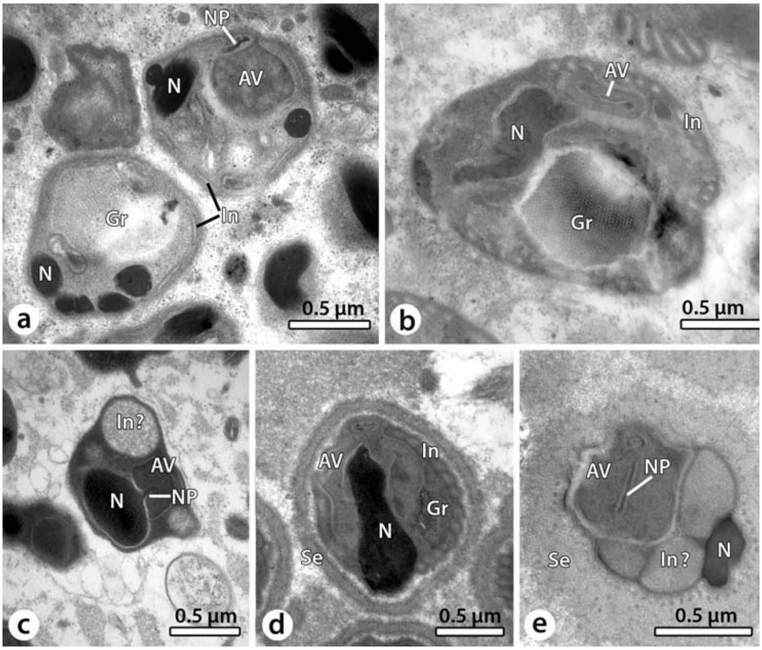

Fig. 4 a: The nucleus of *Hygrobates nigromaculatus* (Hygrobatidae) is elongated and thus sectioned several times; b: The sperm cells of *Piona* cf. *longipalpis* (Pionidae) are provided with a long nucleus and an acrosomal vacuole of a peculiar flat shape, which is sunken into the interior of the cell; c: *Aturus fontinalis* (Aturidae). Rather large electron-lucent inclusions (probably representing modified invaginations) are significant of these sperm cells; d: The spherical sperm cells of *Arrenurus* (*Arrenurus*) *bicuspidator* (Arrenuridae) have distinct peripheral invaginations and are covered by a peculiarly structured secretion; e: Sperm cells of *Arrenurus* (*Truncaturus*) *fontinalis* differ from *A.* (*A.*) *bicuspidator* in showing large lucent inclusions (modified invaginations?). Note also the different thick cover with secretion. Abbr.: AV: acrosomal vacuole; Gr: field of granules; In: invaginations; In?: putative invagination; N: nucleus; NP: nuclear process; Se: secretion.

***Aturus fontinalis* Lundblad, 1920** (Fig. 4c)

Cell shape: almost spherical to slightly irregular; diameter: 1.8 µm

Cell periphery: relatively large, lucent bodies perhaps representing invaginations

Cell interior:

Nucleus: massive, electron-dense with process extending under acrosomal vacuole

Acrosomal complex: large acrosomal vacuole with sac-like extensions; no acrosomal filament

Mitochondria: not evident

Further components: granule field

Arrenuroidea

***Arrenurus* (*Arrenurus*) *bicuspidator* Berlese, 1885** (Fig. 4d)

Cell shape: spherical; diameter: 1.5 µm

Cell periphery: many short invaginations; no complete or very thin secretion layer, instead a thick highly structured cover of secretion (often incomplete)

Cell interior:

Nucleus: massive, homogenous, dense, bean-shaped chromatin body touches the cell periphery; long nuclear process extends under the acrosomal vacuole

Acrosomal complex: sac-like extensions extend from cap-like central part of the acrosomal vacuole; this part with streaks

Mitochondria: several small

Further components: large granule field

***Arrenurus* (*Truncaturus*) *fontinalis* Viets, 1920** (Fig. 4e)

Cell shape: spherical; diameter: up to 1.5 µm

Cell periphery: no invaginations observable, but large lucent inclusions present (probably representing modified invaginations); surrounding thick secretion less structured than in *A.* (*A.*) *bicuspidator*

Cell interior:

Nucleus: homogenous, dense chromatin body, elongated and u-shaped, partly directly below cell membrane bulging the cell periphery; nuclear process extends under the acrosomal vacuole; chromatin body bears several very thin extensions

Acrosomal complex: similar to *A.* (*A.*) *bicuspidator*

Mitochondria: similar to *A.* (*A.*) *bicuspidator*

Further components: large lucent inclusions (see above)

4. Discussion

Sperm cells of Hydrachnidia are aflagellate as are the sperm in all Acari (Alberti 1980a, b, 1991, 2006, Dunlop & Alberti 2008). They are, as in other actinotrichid mites, smaller and of a simpler structure than those of anactinotrichid mites. Nevertheless, there is a great diversity within this limited morphological frame. Until now, the fine structure of spermatozoa of freshwater mites was known from only a few species (Alberti 1980b). Two types had previously been distinguished. Type a) included the sperm cells of *Acerbitas* sp., *Hydrodroma despiciens* (now likely *H. pilosa*), *Sperchon setiger, Limnesia maculata* und *Arrenurus bicuspidator* (with *Sp. setiger* showing a peculiar cell- and nuclear shape) characterised mainly by a large acrosomal vacuole. Type b) was represented by *Limnochares aquatica*, which differs by having a small acrosomal vacuole. Furthermore, the sperm cells of this species are about three times larger than those of the other species. The observations described in the present paper show an even greater diversity reaching beyond these two types. There are lengthy sperm cells with elongated nuclei in *Hydrovolzia, Sperchon* and (nuclei less long) *Sperchonopsis*; irregular or even bizarre cell shapes are seen in the two Sperchontidae; all other species have more or less spherical shapes but the nuclei differ in outline (e.g., spherical: *Limnochares*; sac-like: *Thyas, Hydrodroma, Protzia, Litarachna*; bell-shaped: *Limnesia* or elongated: *Hygrobates* und *Piona*); the acrosomal vacuole may be small and cap-like (*Hydrovolzia*, Eylaoidea) or large (all the other species); all species have acrosomal complexes devoid of an acrosomal filament (which is present in the studied terrestrial Pasasitengona and other Prostigmata; Alberti 1980b). All species have peripheral invaginations, at least they have also been seen in the spermatogenesis of species in which mature spermatozoa do not show this characteristic: *Litarachna, Aturus, Arrenurus* (*Truncaturus*). In some species, mitochondria are numerous and small, in others they are few and large, sometimes they are indistinct. The sperm also differ in the presence and extension of granule fields, which are likely to be accumulations of glycogen. Such granules were found in all species, except for *Litarachna communis*. Such fields presumably represent a fundamental character of hydrachnidian sperm, which was secondarily lost in the marine species. The study of spermatogenesis is likely to complete and improve these preliminary observations.

Comparison with other, i.e. terrestrial, Parasitengona (*Trombidia*; see Alberti 1980b): There are similarities such as peripheral invaginations. The sperm of Trombidia have a small cap-like acrosomal vacuole, which is positioned above a plate-like extension of the elongated nucleus. This plate is penetrated by a long acrosomal filament. Such an arrangement is, except for the acrosomal filament, most similar in *Hydrovolzia*. Thus, these sperm could represent a plesiomorphic type within the Hydrachnidia. The lack of an acrosomal filament, which is a fundamental component of chelicerate sperm (Alberti 2000), and the field of granules (not yet seen in Trombidia) may be regarded as synapomorphies of Hydrachnidia.

Eylaoidea: The large sperm cells of *Limnochares aquatica* are certainly derived. Spermatozoa of *Eylais rimosa* also possess a small acrosomal vacuole and thus show an intermediate structure between *Limnochares* and the more basal type represented by *Hydrovolzia placophora* or the Trombidia. Protziidae were originally placed together with Limnocharidae and Eylaidae by Viets (1936) (forming his Limnocharae). However, the sperm cells of *Protzia* are provided with a large acrosomal vacuole, which is characteristic of all the other investigated Hydrachnidia. This observation supports the separation of Protziidae from

Eylaoidea (e.g., Cook 1974, Kethley 1982, Smith & Cook 2001, Davids et al. 2006, who classified these mites as subfamily Protziinae within Hydryphantidae) and simultaneously demonstrates the validity of spermatological characters.

Other Hydrachnidia: These are characterised by their large acrosomal vacuole and the narrow nuclear process, characters supporting the taxon Euhydrachnidia (Witte & Döring 1999, Wohltmann et al. 2001). There are two types of acrosomal vacuoles within this group. Most species have a voluminous, sac-like acrosomal vacuole which often shows peculiar streaks as a substructure (*Protzia eximia* included!). Only *Piona* has a flat acrosomal vacuole with a different position. According to the present state of knowledge, this character divides taxa composing the Hygrobatoidea, which seem to be most heterogenous. However, this impression may be a consequence of the fact that only a few or single species have yet been investigated from the other superfamilies. Since the shape of the acrosomal vacuole is quite characteristic and certainly represents a derived character, these differences seem nevertheless remarkable. Furthermore, the differences between the two *Arrenurus* species seem to be worth mentioning since they might support their classification within two different subgenera.

Position of Pontarachnidae: Pontarachnidae are mostly classified with the Hygrobatoidea (as was done in this paper). Tuzovskij (1983, 1987) suggested these marine mites to present a superfamily Pontarachnoidea of its own. Sperm structure shows indeed peculiarities with regard to acrosomal vacuole, peripheral invaginations and the field of granules, which is apparently lacking. This could support the view of Tuzovskij. However, as already stated, the Hygrobatoidea seem to be quite heterogenous (see, e.g., *Piona*) and thus a definite conclusion needs far more species to be investigated.

Concluding remarks: Certainly, these spermatological observations are still in a preliminary state. However, our results do not question the monophyly of Hydrachnidia. The monophyly of Euhydrachnidia also seems to be supported. Of course, it would be desirable to broaden these studies taking more taxa into account, including Stygothrombioidea. Furthermore, investigating spermatogenesis in detail would help to understand characters better. Such studies almost certainly will reveal further peculiarities useful for phylogenetic-systematical interpretations. Finally, such future studies should reveal correlations with peculiarities in reproductive biology (e.g., modes of sperm transfer: e.g., Böttger 1962, Schaller 1979, Witte 1991, Proctor 1992; peculiarities of female genital systems, egg shells etc.: e.g., Alberti & Coons 1999) and thus may contribute to a better understanding of evolutionary processes (e.g., Witte & Döring 1999, Wohltmann et al. 2001, Alberti & Michalik 2004). The remarkable variety of secretions found in the male genital tract also seems to be a topic worth studying in detail.

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6. References

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